NEURAL CONTROL OF GAIT IN PEOPLE WITH HAEMOPHILIC ARTHROPATHY

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NEURAL CONTROL OF GAIT IN PEOPLE WITH HAEMOPHILIC ARTHROPATHY

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“A winner is a dreamer who never gives up”

Nelson Mandela

LIST OF ABBREVIATIONS

a.u. Arbitrary unit

BF. Biceps femoris

CG. Control subjects

CNS. Central nervous system

EMG. Electromyography

GMAX. Gluteus maximus

GMED. Gluteus medius

HJHS. Haemophilic Joint Health Score

LG. Lateral gastrocnemius

MG. Medial gastrocnemius

NNMF. Non-negative matrix factorization

OA. Osteoarthritis

PWHA. People with haemophilic arthropathy

RF. Rectus femoris

ROM. Range of motion

SOL. Soleus

SPM. Statistical parametric mapping

ST. Semitendinosus

TA. Tibialis anterior

tVAF. Total variance accounted for

tVAF1. Total variance accounted for one synergy

VAS. Visual Analogue Scale

VL. Vastus lateralis

VM. Vastus medialis

Walk-DMC. Walking Dynamic Motor Control

TABLE OF CONTENTS

FOREWORD

Haemophilia is a bleeding disorder caused by a deficiency of coagulation factors VIII or factor IX. People with severe haemophilia may have spontaneous bleeding events or bleed in response to minor trauma; most of the events occur in the joints and muscles. The repetitive intraarticular bleedings in people with haemophilia results in irreversible joint damage, known as haemophilic arthropathy. Haemophilic arthropathy is characterized by joint impairment, chronic pain and reduced quality of life. The standard treatment to prevent the bleeding events is the prophylactic treatment, in which a replacing clotting factor is administered routinely several times a week. In Chile (South America), where all data for this thesis were collected, the treatment with the replacing clotting factor for people with haemophilia was included in the Explicit Health Guarantees in 2006, which include a set of benefits guaranteed by law allowing access, opportunity, financial protection, and quality of care. The prophylactic treatment was guaranteed only for people with haemophilia under 18 years. The incorporation of adults into the prophylaxis was done gradually over the years. Due to the late use of prophylaxis, many adults over 30 years currently have severe musculoskeletal sequelae (muscle atrophy and joint contracture), which affect the essential activities of daily living such as walking and their quality of life. Some have already undergone total hip, knee, and ankle replacement, and others are currently waiting for orthopedic surgery.

In my work as a physical therapist, I have had the opportunity to treat many persons with haemophilic arthropathy and severe functional limitations. The greatest

challenge has been to recover the gait pattern towards normal, often after total joint replacement. One of the main barriers was the long-term history of an altered gait pattern. Collecting data in Chile for this PhD project has been an opportunity to understand the effects of haemophilic arthropathy on the neural control of gait, especially in those patients with a long history of joint damage and joint contracture. In my thesis, I have applied new tools to monitor neural control of gait with manageable and rapid interpretation. I hope these tools will find their way into clinical practice and help generate new treatments to improve functionality and quality of life for people with haemophilic arthropathy.

ABSTRACT

Haemophilia is a bleeding disorder caused by a deficiency of coagulation factors VIII or factor IX. People with severe haemophilia may have spontaneous bleeding events or bleeding in response to minor trauma; most of the events occur in the joints and muscles. The most frequent clinical manifestation is haemophilic arthropathy, which results from repetitive intraarticular bleeding and inflamed synovial membrane, which may result in chronic pain and joint impairment. The overall aim of my thesis was to investigate the neural control of gait in people with haemophilic arthropathy (PWHA). The findings of my thesis indicate that neural gait control is affected in PWHA, and the changes in the neural control of gait are associated with joint damage, pain and chronicity of the joint constraint. My thesis gives a new perspective on how to monitor disease progression in PWHA—providing new perspectives to improve the therapeutic interventions that aim to recover gait in PWHA.

CHAPTER 1

GENERAL INTRODUCTION

Haemophilia is more than a bleeding disorder

Haemophilia is an X chromosome-linked bleeding disorder that affects the blood's ability to clot caused by a deficiency of coagulation factors VIII (haemophilia A) or factor IX (haemophilia B) (Oldenburg et al. 2004). Most people who have haemophilia are male. The classification of disease severity is based on the amount of residual factor VIII or factor IX activity (severe < 1%, moderate 1-5%, and mild >5% and<40% of factor activity in the blood) (Berntorp et al. 2021; Blanchette et al. 2014). Male with severe and moderate haemophilia may have spontaneous bleeding events or bleed in response to minor trauma; most of the events occur in the joints and muscles (Berntorp et al. 2021). Compared to males, females are usually heterozygous carriers of one mutated gene and may have reduced factor VIII or factor IX levels, usually associated with mild symptoms. To stop bleeding events, the usual treatment consists of an infusion (into the vein) of the concentrate of the deficient coagulation factor (factor VIII or factor IX) (Berntorp et al. 2021). However, access to treatment is restricted in several countries due to its high cost (Moreno and Cuesta-Barriuso 2019). There are two types of treatment modalities: on-demand and prophylaxis. On-demand is defined as treatment accessed if there is an acute event. Prophylaxis (or standard care) is the regular replacement therapy, in which a replacing clotting factor is administered routinely

several times a week or other products with a long half-life are administered one time a week to prevent bleeding events and musculoskeletal complications (Moreno and Cuesta-Barriuso 2019; Srivastava et al. 2020). The prevalence of haemophilia is low (haemophilia A, 12.8 per 100,000 males; haemophilia B 1.6 per 100,000 males) (Stonebraker et al. 2012), but the care costs are high. For instance, among younger adults, bleeding-related non-pharmacy costs (i.e., number of outpatient visits, hospitalization days, emergency room visits) for patients without prophylaxis can be higher than 50.000 euros per year (Shrestha et al. 2017).

The major morbidity in haemophilia is haemophilic arthropathy (HA), which results from repetitive intraarticular bleeding and inflamed synovium, generating irreversible changes in cartilage and bone, with the subsequent ligament and muscle impairments (Figure 1) (van Vulpen et al. 2018).

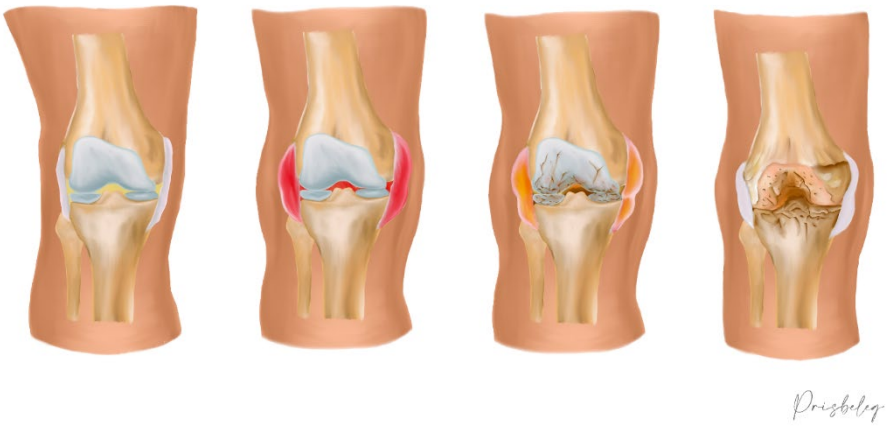


Figure 1. A) Schematic figure of haemophilic arthropathy which results from repetitive intraarticular bleeding and inflamed synovium, generating irreversible changes in cartilage and bone, with the subsequent ligament and muscle impairments.

HA may generate a disabling condition that can affect joint function and cause pain, affecting the quality of life (Hilberg et al. 2001; Krüger et al. 2018; Ucero-Lozano et al. 2021). Inducing a single hemarthrosis event in a rat model resulted in synovitis within 24 hours and cartilage and bone pathology 48-96 hours after the event (Christensen et al. 2019). This indicates that joint impairment may result from a single hemarthrosis event. Without access to the treatment aimed to stop bleeding events in the joints, the process of joint deterioration in haemophilic arthropathy is progressing at a higher rate than in osteoarthritis and rheumatoid arthritis (Pulles et al. 2019). It is well known that about 90% of people with severe haemophilia experience arthropathy in the ankles and knees by the second or third decade of life (JONES 1958; Liu et al. 2020). The reduced range of motion (ROM), lower muscle strength, and altered walking patterns are the most remarkable changes in the lower limb in people with haemophilic arthropathy (PWHA) (Stephensen et al. 2012). Haemophilic arthropathy can vary in severity, from asymptomatic joint damage to severe sequelae such as knee flexion contracture and multijoint joint impairment (Figure 2). The levels of joint damage depend mainly on how early the prophylaxis was started and the access to on-demand treatment to stop intra-articular and muscular bleeding episodes.

Figure 2. Patient with advanced stage of haemophilic arthropathy, characterized by knee flexion contracture.

Clinical assessment of PWHA is usually performed by examining joints and muscles, applying a Haemophilic Joint Health Score (HJHS), and visual examination of posture and gait (Stephensen et al. 2019). The HJHS includes the domain of body

structure and function (i.e., impairment) of the most affected joints (knees, ankles and elbows), and global gait assessment (i.e., capacity to walk, up-down stairs, running, hopping on one leg) (Hilliard et al. 2006). The HJHS is used to assess the degree of severity. For example, if one joint or multiple joints are involved.

In addition, several tools have been proposed to monitor joint deterioration, such as radiographic, ultrasound and magnetic resonance imaging scores of joints (Daffunchio et al. 2021; Pasta et al. 2020; Silva et al. 2008; Spasov et al. 2020). The laboratory gait analysis and surface EMG have also been proposed to assess joint impairment and neuromuscular control in PWHA (Kurz et al. 2012; Lobet et al. 2010; Pasta et al. 2020; Seuser et al. 2018; Stephensen et al. 2012). These analyses provide insight about the mechanical and neuromuscular adaptations to arthropathy. However, its use as a clinical tool is limited, probably because normative values are lacking and/or the results are difficult to interpret for clinicians.

A BRIEF HISTORY OF HAEMOPHILIA

There is evidence that the first modern description of haemophilia appeared in 1803 when the American physician Dr. John Conrad Otto described it as an inheritable bleeding disorder in several families in which only males were affected ("bleeders") and transmission occurred via unaffected females (Schramm 2014). However, the word haemophilia, which means "affinity to blood", appears to have been documented for the first time in 1828 in Switzerland at the University of Zurich by Dr. Johann Lukas Schönlein, who described the condition in his dissertation entitled "*About haemophilia or the hereditary predisposition to fatal bleeding*" (Schramm 2014). In 1890, Dr Franz König, an orthopedic surgeon,

described for the first time the link between haemophilia and the development of joint disease characterized by chronic joint contractures (Ingram 1976).

Blood transfusion, as proposed by Schönlein in 1832, was the first treatment to stop bleedings (Schramm 2014). A century later (in the mid-1960s), the clotting factor concentrate derived from plasma was developed, opening a new treatment, and improving the quality of life and life expectancy (Schramm 2014). Prophylactic treatment began in Sweden in the 60s (Nilsson et al. 1992). Between 1994 and 1996, the World Federation of Haemophilia, together with Canadian and Dutch medical organizations recommended prophylaxis in patients with severe haemophilia (Moreno and Cuesta-Barriuso 2019). However, in several underdeveloped and developing countries, such as in Latin America (Boadas et al. 2018), the access to factor concentrates is still limited due to the high costs (Moreno and Cuesta-Barriuso 2019). Prophylactic treatment is currently considered the only way to prevent bleedings (Srivastava et al. 2020). Due to limited access to it, several people with haemophilia have severe musculoskeletal sequelae and impaired quality of life.

CURRENT (GAPS IN) KNOWLEDGE IN THE NEURAL CONTROL OF GAIT IN HAEMOPHILIC ARTHROPATHY

Previous research has focused predominantly on the musculoskeletal aspects of haemophilia, with a few studies addressing the potential effects of arthropathy on the central nervous system (CNS). Haemophilia was shown to result in altered proprioception and pain processing (Cruz-Montecinos et al. 2017; Hilberg et al. 2001; Krüger and Hilberg 2020) and increased co-contraction during standing conditions (Kurz et al. 2011, 2012). However, it is unknown how haemophilic

arthropathy affects neuromuscular control during dynamic tasks such as walking. Furthermore, it is unknown how levels of arthropathy severity affect the neural control of gait.

Investigating if neural control of gait is affected in PWHA is relevant since increased co-contraction around joints may increase the intraarticular load (Hodges et al. 2016; Shelburne et al. 2006). Electromyography (EMG) has been widely used to examine neural control during gait in several musculoskeletal and neurological diseases such as osteoarthritis, cerebral palsy and stroke (Bekius et al. 2020; Van Criekinge et al. 2020; Mills et al. 2013a). However, assessment of neural control of gait has only been minimally studied in PWHA. This assessment may help advance our understanding in what way the changes in neural control of gait are associated with joint damage and joint constraints, especially in those PWHA with long-term exposure to joint damage and joint contractures (Atilla et al. 2012; Heijnen and De Kleijn 1999; Nelson et al. 1989).

NEURAL CONTROL OF GAIT AND MUSCLE SYNERGY ANALYSIS

What is neural control of gait?

Understanding the mechanisms of the neural control of movement for vertebrate and non-vertebrate animals is still one of the challenges in science. Automatic rhythmic movements (e.g., locomotion, mastication, and breathing) are the most studied motor behaviours. The cyclical muscle patterns needed for walking, and other rhythmical activities, are generated by spinal neural networks (Duysens and Van De Crommert 1998). To generate adequate coordination across all muscles, feedback from joints, muscles, tendons, and skin is essential (Frigon et al. 2022; Zehr 2005). One of the main challenges of the CNS is to control a large number of

muscles during movement. This has been called the problem of abundance, which considers that there are multiple or redundant movement solutions to achieve the same task or goal result (Carpenter 1968). One possible solution that the CNS may use to solve the abundance problem is generating movements by exiting a group of muscles in patterns (called muscle synergies) instead of by exciting muscles individually (Bizzi et al. 1991; Tresch et al. 2006; Tresch and Jarc 2009). In this way, the CNS uses flexible combinations of a few muscle synergies to produce a wide range of motor behaviours (walking, jumping, flying, swimming, etc.)(Ting and McKay 2007).

During locomotion in humans and in other vertebrates (cats, monkeys, rats, and guinea fowl), four or five basic muscle synergies have been identified from EMG recordings of several leg and trunk muscles (Dominici et al. 2011; Ivanenko et al. 2004). Despite their phylogenetic and morphological distances, these common locomotion synergies among vertebrates are presumed to stem from primitive patterns related to common ancestral neural networks (Dominici et al. 2011). Muscle synergies can be characterized by EMG activities of muscles represented by combinations of time-independent weights (also called motor modules) and time-dependent coefficients (motor primitives) (Chvatal and Ting 2012; Tresch and Jarc 2009). These synergies represent the modular organization (spatiotemporal structure) for different motor tasks (Chvatal and Ting 2012; Santuz et al. 2019). The muscle synergies during gait have been extensively studied in healthy individuals and in people with neurological diseases (Bekius et al. 2020; Cherni et al. 2021; Van Criekinge et al. 2020; Ivanenko et al. 2004; Janshen et al. 2017). However, less is known about the modular organization in people with musculoskeletal diseases (Kubota et al. 2021; Roelker et al. 2021).

How can we measure the neural control of gait?

The assessment of activity patterns of single muscles in the leg has been used to assess how neuromuscular control is adapted to changes of joint load during locomotion (Lay et al. 2007; Sinkjær et al. 2000; Wall-Scheffler et al. 2010). Instead of focusing on single muscles, muscle synergy analysis has been applied to study the modular organization. To study modular organization, EMG data are decomposed into motor modules and motor primitives(Kubota et al. 2021; Santuz et al. 2019; Tresch and Jarc 2009).

Different methods have been used to decompose the EMG data of which the non-negative matrix factorization (NNMF) is one the most used methods (Rabbi et al. 2020; Tresch et al. 2006). NNMF constrains the EMG data into non-negative vectors, which contain the motor modules (W) and motor primitives (C) (Figure 2). The product of W and C should approximate the original EMG data. The product of W and C is used then to find the combination that best describes the original data variance (Rabbi et al. 2020). The total variance accounted for (tVAF) is typically employed to quantify reconstruction accuracy, by linearly combining motor modules and motor primitives to reconstruct the original EMG data (Rabbi et al. 2020; Tresch et al. 2006). Four-to-five different muscle synergies have been identified to represent 90 % of tVAF of lower extremity muscle activation patterns during locomotion in healthy individuals (Figure 3).

The number of muscle synergies, the structure of motor modules, the pattern of motor primitives and the tVAF, are commonly used to describe the complexity of neuromuscular control. The tVAF value for one synergy (tVAF1) has also been used

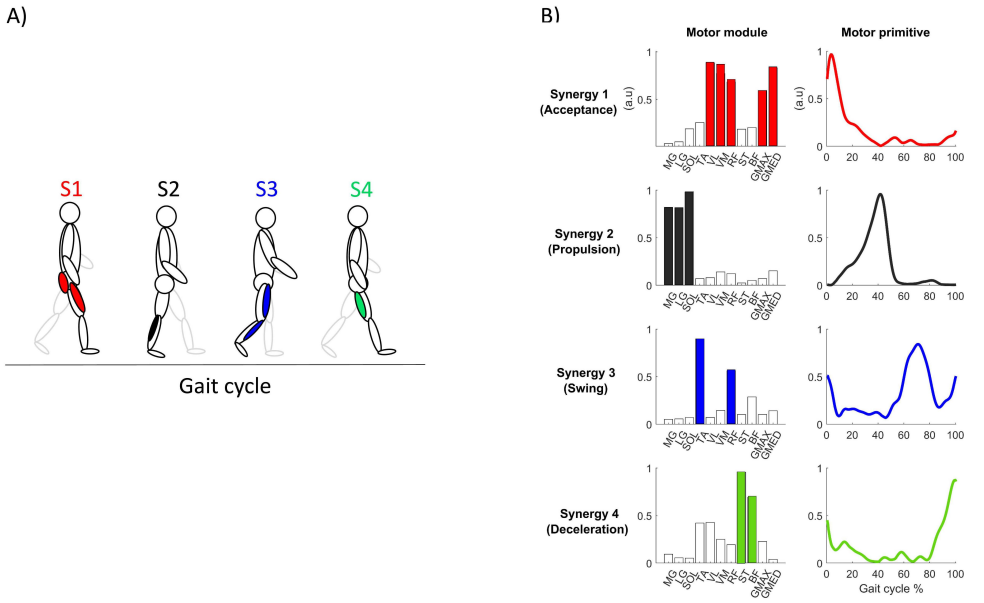


Figure 3. Schematic representation of muscle synergies across gait cycle in healthy adults. **A)** The red color represents the principal muscle contributions of each synergy (S). *S1 (red), acceptance* (with a principal contribution of hip extensors/abductors and knee extensors) is exited during early stance. *S2 (black), propulsion* (with a principal contribution of ankle plantar flexors) is exited during late stance. *S3 (blue), swing* (with a principal contribution of ankle dorsiflexors and biarticular knee extensors) is exited during early swing. *S4 (green), deceleration* (with a principal contribution of hamstring muscles) is exited during late swing. B) Schematic of motor modules and motor primitives during gait.

as a summary metric of such complexity. A higher value is found with, higher levels of co-activation between synergistic and antagonist muscles and is interpreted as a lower neural complexity (Schwartz et al. 2016; Steele et al. 2015). In 2015, Steele et al. proposed a new tool to assess the neural control of gait called the Walking

Dynamic Motor Control (Walk-DMC) index, which is based on the z-score of the VAF1 from the healthy control group (Steele et al. 2015). Compared to synergy analysis, the Walk-DMC index is a metric that clinicians can interpret more quickly, making the identification of patients with altered neural control of gait more accessible.

GENERAL AIM AND OUTLINE

The overall aim of my thesis was to investigate the neural control of gait in PWHA. The specific aims were:

i. To assess changes in EMG activity patterns of single muscles.
ii. To assess changes in modular organization of gait.
iii. To investigate the association between clinical measures of musculoskeletal function and measures of modular organization.
iv. To elucidate the mechanisms driving the changes in neural control.

I hypothesized that neural gait control is affected in PWHA, and the changes in neural control of gait are associated with joint damage and chronicity of the joint constraint. Gait was selected because the knees and ankles are the most prevalent affected joints in adults PWHA. The core of my thesis is investigating neural control by studying EMG activity patterns of single muscles and/or muscle synergies, as well as their interaction with joint kinematics and clinical outcomes. For this purpose, EMG activity of muscles crossing hip, knee and ankle as well as the lower limb kinematic, and clinical measures (HJHS, pain, walking velocity) were assessed.

In **Chapters 2, 3 and 5,** the changes in neural control of gait in PWHA with various levels of arthropathy severity were assessed using the synergy analysis. In **chapter 4**, the neural control of individual muscles was assessed. In **chapters 2 and 3**, the complexity of neuromuscular control was assessed using the Walk-DMC index. In **chapter 5**, possible merging of muscle synergies was evaluated to investigate the effects of short-term and long-term exposure to knee joint constraints on the modular organization of gait.

Chapter 2 investigates the changes in the complexity of neuromuscular control during gait in PWHA with varying levels of arthropathy severity compared to a healthy control group. This chapter described for the first time the effect of haemophilic arthropathy on neuromuscular control of gait.

Chapter 3 investigates the association between clinical outcomes (visual analogue scale, the degree of knee flexion contracture and HJHS) and Walk-DMC in PWHA with varying levels of arthropathy severity. In addition, the clinical outcomes of PWHA with normal and altered neural control of gait were compared.

Chapter 4 investigates the changes in the neural control of individual muscles and joint kinematics in PWHA with mild joint restriction compared with healthy controls using statistical parametric mapping on the time series.

Chapter 5 assessed the effects of short-term (minutes) and long-term exposure (years) to knee joint constraints on modular organization of gait. In this chapter, we investigate how the CNS responds to a knee joint constraint in healthy controls (short-term) and has adapted in PWHA with long-term exposure to a severe joint constraint.

In chapter 6, a summary is provided and the main findings of my thesis are discussed, focused on clinical, physiological, and methodological perspectives.

CHAPTER 2

Neuromuscular control during gait in people with haemophilic arthropathy

Introduction: Effects of haemophilic arthropathy on neuromuscular control during gait are currently unknown. **Aims:** (a) To assess how haemophilic arthropathy affects the complexity of neuro-muscular control during gait; (b) To investigate the relationship between complexity of neuromuscular control and joint impairment. **Methods:** Thirteen control subjects (CG) walked overground at their preferred and a slow velocity and thirteen people with haemophilic arthropathy (PWHA) walking at their preferred velocity. Surface electromyography (EMG) was collected from eleven leg muscles. Electromyography variance explained by muscle synergies (sets of co-activated muscles that can be recruited by a single signal) was calculated by the total variance accounted (tVAF). Three measures were used to evaluate complexity of neuromuscular control: (a) the number of synergies required for tVAF > 90%, (b) tVAF as a function of the number of muscle synergies, and (c) the dynamic motor control index (Walk-DMC). Impairment of ankle and knee joints was determined by the Haemophilia Joint Health Score (HJHS). **Results:** The same number of the muscle synergies was found for each group ($P > 0.05$). For both walking velocities tested, tVAF1 was higher in PHWA ($P < 0.05$). The Walk-DMC of PWHA was lower than that of the CG for both walking velocities ($P < 0.05$). For PWHA, no significant correlation was

found between HJHS (sum knee and ankle) and Walk-DMC index (r = −0.32, P = 0.28). **Conclusions:** These results indicate differences between PWHA and CG in the neuromuscular control of gait. The Walk-DMC and tVAF1 may be useful measures to assess changes in neuromuscular control in response to treatment.

Cruz-Montecinos C, Pérez-Alenda S, Cerda M, Maas H. Neuromuscular control during gait in people with haemophilic arthropathy. Haemophilia. 2019 Mar;25(2): e69-e77. *doi: 10.1111/hae.13697.*

INTRODUCTION

Haemophilic arthropathy is the result of repetitive intra-articular bleeding and synovial inflammation (van Vulpen et al. 2017). In the lower limb, haemophilic arthropathy commonly affects the range of motion of the knee and ankle joints and has also an impact on muscle size, muscle force capacity and proprioception (Hilberg et al. 2001; van Vulpen et al. 2017). While the interest in the musculoskeletal properties and biomechanics of movement of people with haemophilic arthropathy (PWHA) has recently increased (Cruz-Montecinos et al. 2019b; Lobet et al. 2012, 2018; Seuser et al. 2018; Suckling et al. 2018), the effects of haemophilic arthropathy on neuromuscular control during gait have not been investigated.

Instead of focusing on the activity patterns of individual muscles, the neuromuscular control of motor tasks can be described by muscle synergies (D'Avella et al. 2003; Ting and McKay 2007; Tresch et al. 1999). A muscle synergy is a group of muscles that are recruited simultaneously (i.e co-activated) with distinct relative levels of activation. The central nervous system (CNS) is presumed to produce movements not by activating individual muscles, but by activating muscle synergies (D'Avella et al. 2003; Ting and McKay 2007; Tresch et al. 1999). In healthy individuals, it has been shown that a large part (>90%) of the variance in muscle activity during gait can be described by a limited number (4-5) of muscle synergies (Ivanenko et al. 2004; Neptune et al. 2009). The same approach has been applied in several neurological diseases (i.e cerebral palsy, Parkinson, stroke and incomplete spinal cord injury). Patients were found to use a lower number of muscle synergies and altered structure of the synergies, which was related to functional and clinical assessments

(Barroso et al. 2016; Clark et al. 2010; Steele et al. 2015; Taborri et al. 2018). A lower number of synergies is interpreted as a more simplified (i.e decreased complexity) control by the CNS. When applied in musculoskeletal diseases (i.e sacroiliac joint pain, anterior cruciate ligament-deficient, gluteal tendinopathy), also a different synergy structure compared to a control group was observed, but no difference in the number of muscle synergies (Allison et al. 2018; Feeney et al. 2018; Serrancolí et al. 2016).

Recently, a new measure has been proposed called the Walking Dynamic Motor Control Index (Walk-DMC), which is based on the total EMG variance explained by one synergy (Steele et al. 2015). The Walk-DMC has been proposed as a potential metric to assess the complexity of motor control and was shown to be associated with clinical outcomes after conservative treatment and orthopaedic surgery in patients with cerebral palsy (Shuman et al. 2016, 2018; Steele et al. 2015). Applying measures of complexity of neuromuscular control may also be a valuable clinical tool to assess the level of neuromuscular impairment in PWHA with different levels of joint damage.

The aims of this study are as follows: (a) to assess how haemophilic arthropathy affects the complexity of neuromuscular control during gait, (b) to investigate the relationship between complexity of neuromuscular control and joint impairment.

MATERIAL AND METHODS

Participants

This study was approved by the local ethical committee and conducted in

agreement with the Declaration of Helsinki. All participants were informed about the purpose and procedures of the project and gave their written informed consent to participate in the study. Based on non-probability sampling, thirteen PWHA were recruited in two hospitals in Santiago (Chile), and thirteen healthy control subjects (student and employees) were recruited from the University of Chile (for their characteristics see Table 1).

Table 1. Basic characteristics of the two groups

Variables	**CG (n=13)**	**PWHA (n=13)**	**p-value**
Age (years)	28.4 ± 6.2	28.7 ± 6.9	0.906
Body mass (Kilograms)	75.5 ± 8.1	74.4 ± 8.7	0.280
Height (centimetres)	176 ± 0.04	171 ± 0.10	0.092
Body mass index	24.4 ± 1.9	25.3 ± 2.8	0.450
Pain during walk (VAS 0-10)	0 [0 0]	1 [0 6]	0.019*
Preferred velocity in 30 Meters (m/s)	1.2 ± 0.2	1.0 ± 0.2	0.016*
Physical activity (>150 min/week)	7/13	4/13	0.223
PWHA were diagnosed with Haemophilia A	N.A.	13/13	N.A.
Severity of Haemophilia (severe)	N.A.	10/13	N.A.
Severity of Haemophilia (moderate)	N.A.	3/13	N.A.
Evaluated limb			
HJHS ankle (points)	N.A.	7.1 ±3.3	N.A.
HJHS knee (points)	N.A.	5.8 ± 4.9	N.A.
Sum HJHS knee and ankle (points)	N.A.	12.9 ± 6.0	N.A.
Contralateral limb			
HJHS ankle (points)	N.A.	5.2 ± 4.8	N.A.
HJHS knee (points)	N.A.	3.8 ± 5.0	N.A.
Sum HJHS knee and ankle (points)	N.A.	9.1 ± 8.9	N.A.

Parametric distribution: Mean ± SD. Nonparametric distribution: Median [Range]. CG, control group; HJHS, Haemophilia Joint Health Score; NA, Not applicable; PWHA, people with haemophilia; VAS, Visual Analogue Scale. *P-value <0.05.

Inclusion criteria for PWHA: Males, diagnosed with haemophilia A or B, severe or moderate (severe <1% and moderate 1%-5% of normal factor activity in blood), haemophilic arthropathy with a minimum of two points (sum knee and ankle in evaluated limb) of the Haemophilia Joint Health Score (HJHS), over 18 years of age and under 45 years, prophylaxis treatment with deficient factor (i.e VIII or IX), and body mass index lower than 30. Exclusion criteria: History of hip, knee or ankle

arthroplasty in the evaluated limb, equinus foot, incapacity to walking independently, history of muscle or joint bleeding in lower limbs in the last 2 months, chronic cardiac and/or respiratory pathology and neurological disease.

Inclusion criteria for control subject: Males over 18 years of age and under 45 years, no haemophilia and body mass index lower than 30. Exclusion criteria: Scoliosis, history of acute or chronic musculoskeletal disorders, cardiac and/or respiratory pathology and neurological disease.

Data acquisition

In PWHA, the limb with the highest score on the HJHS was selected. In the control group (CG), the dominant limb was assessed, which was determined by asking the subjects which leg they would use to kick a ball (Chia Bejarano et al. 2017). After shaving and cleaning the skin with alcohol, surface electrodes (Ag–AgCl, Kendall H124SG) were placed (interelectrode spacing 2 cm) on the following muscles according to SENIAM guide-lines (Hermens et al. 2000): Medial Gastrocnemius (MG), Lateral Gastrocnemius (LG), Soleus (SOL), Tibialis Anterior (TA), Vastus Lateralis (VL), Medialis (VM), Rectus Femoris (RF), Semitendinosus (ST), Biceps Femoris (BF), Gluteus Maximus (GMAX) and Gluteus Medius (GMED). Muscle activity patterns were assessed using a wireless EMG system (MyoSystem DTS; Noraxon USA Inc, Scottsdale, CA, USA), with a sampling rate of 1500 Hz. Gait cycle events were detected by a synchronized wireless pressure sensor placed underneath the heel of the foot.

Experimental protocol

To assess if subjects had a sedentary lifestyle (<150 minutes per week of moderate physical activity), they were asked to indicate how many minutes per week they were involved in physical activity (Bennett et al. 2006). Each subject was invited to walk barefoot overground at their preferred velocity and the CG also walked at a slower velocity similar to that of the mean preferred velocity in PWHA (1.0 m/s). Each velocity was practiced three times for 10 m. Mean velocity was assessed by dividing total distance by total time. Subsequently, each subject walked barefoot for 30-m, two times in each velocity, with 2 minutes of rest in between tests. Between 1 and 2 hours prior to the experiment, patients received prophylactic treatment.

EMG data analysis

For the EMG and synergies analysis, Matlab software was used (MathWorks, Inc, Natick, MA, USA). For each condition in each group, 20 gait cycles were included for the analysis. A bandpass filter (20-500 Hz) followed by rectification using Hilbert transformation was applied. Subsequently, EMG signals were low-pass filtered at 10 Hz and per condition normalized to the maximum value of all included cycles (Cappellini et al. 2006; Martino et al. 2015). Then, EMG data was time normalized to 200 points (Cappellini et al. 2006). Non-negative matrix factorization (NNMF) was used to extract muscle synergies from the EMG signals (for a comprehensive description see Tresch et al. 2006). Briefly, the EMGs were combined into an m × t matrix, where m represents the number of muscles (11 in this study) and cycles (20 cycles), and t is the time base (200 points). The NMMF results in the muscle weightings (i.e contribution) for each synergy (W) and the matrix encoding the

activation pattern of each synergy (C). Note that the product of W and C should approximate the original EMG data. The NNMF algorithm was iterated 20 times for each number of synergies between 1 and 4, and the iteration with the lowest reconstruction error was selected. The difference between reconstruction of EMG data and the original EMG data was calculated using the total variance accounted for (tVAF) (Table 2, Equation 1).13 The tVAF was calculated for an increasing number of synergies (from 1 to 4). The number of synergies was increased until tVAF was >90% or until adding another synergy did increase tVAF by <5% (Clark et al. 2010). We used three measures to evaluate complexity of neuromuscular control (Shuman et al. 2017; Steele et al. 2015): (a) the number of synergies required for tVAF > 90%, (b) tVAF as a function of the number of muscle synergies and (c) Walk-DMC index. The Walk-DMC is a z-score based upon tVAF1, using the average and standard deviation of tVAF1 from the healthy CG (Table 2, Equation 2)(Steele et al. 2015). A higher tVAF1 results in a lower Walk-DMC score (see Figure 1).

Table 2. Equations used for the muscle coordination analysis.

Index	Equations
$1.\ tVAF$	$\left(\frac{\left[\sum_j^t \sum_i^m (EMGr - EMGo)^2\right]}{\left[\sum_j^t \sum_i^m (EMGo)^2\right]}\right)$
$2.\ walk - DMC$	$100 + 10\left[\frac{tVAFAVGc - tVAF1}{tVAFSDc}\right]$

The total variance accounted for (tVAF). tVAF by one synergy (tVAF1). Original EMG data (EMGo). Reconstructed EMG data (EMGr). Dynamic motor control index (Walk-DMC). Average (AVGc) and standard deviation (SDc) of tVAF1 from the control group.

Clinical assessments for PWHA

To assess the intensity of pain (scale 0-10 points) during walking barefoot, the Visual Analogue Scale (VAS) was applied. The HJHS 2.1 score is used to assess joint health status in both knees and ankles(Hilliard et al. 2006).

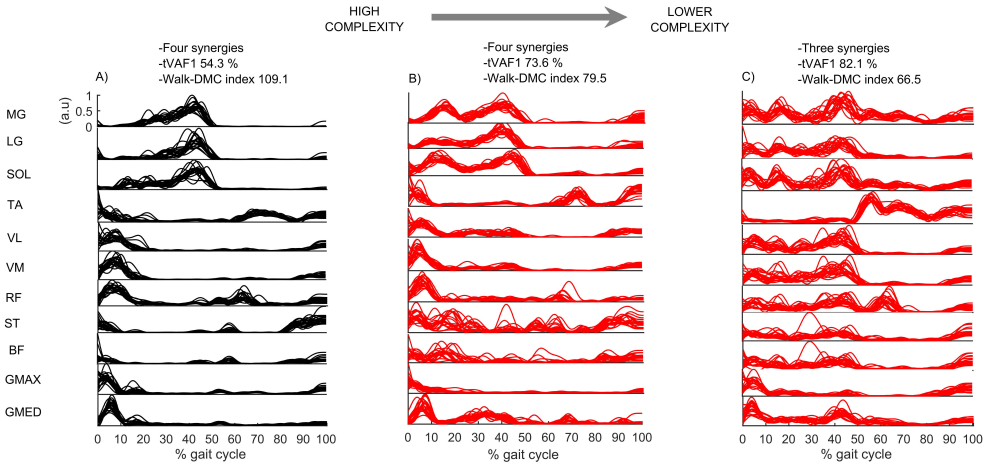


FIGURE 1 EMG activity patterns of multiple muscles during gait. A, Example of one healthy individual (black). EMG activity patterns during gait of two people with haemophilic arthropathy (PWHA) (red). B, Example of PWHA with low HJHS score in both knee and ankle (knee = 0 points; ankle = 2 points). C, Example of PWHA with high HJHS score in both knee and ankle (knee = 8 points; ankle = 8 points). The data show 20 gait cycles during slow velocity walking for the healthy subject (1.2 m/s) and the preferred velocity of the haemophilic patients (1.24 m/s and 1.03 m/s, respectively). Note that in PWHA, the EMG activity patterns of several muscles are similar indicating a high degree of co-activation between flexion and extension muscles. In the PWHA with high HJHS score (C), more than 80% of variance of all muscles can be explained by one synergy, while in the healthy subject one synergy explains only 54% of the variance. This can be explained by more co-activation between flexion and extension muscles and has been interpreted as an indication of more simplified control by the central nervous system. a.u., Arbitrary unit; BF, Biceps Femoris; GMAX, Gluteus Maximus; GMED, Gluteus Medius; LG, Lateral Gastrocnemius, MG, Medial Gastrocnemius; RF, Rectus Femoris; TA, Tibialis Anterior; SOL, Soleus; ST, Semitendinosus; VL, Vastus Lateralis; VM, Medialis. The total variance accounted for one synergy (tVAF1). The dynamic motor control index (Walk-DMC).

Statistical analysis

For all statistical analysis, Matlab software was used (MathWorks, Inc., Natick, Massachusetts, United States). The alpha-level was set at 0.05. The normality of data was evaluated through the Shapiro-Wilk test. Data is expressed as the mean ± standard deviation.

Two assessments were made for all variables: 1) the comparison of CG during preferred walking velocity (CG-pref) with PWHA, 2) and a comparison of CG during slow velocity (CG-slow) with PWHA.

The Chi-square test was used to compare the number of muscle synergies for tVAF > 90% between groups. To evaluate differences in the tVAF as a function of number of synergies, two-way repeated measures ANOVA (number of synergies × group) was used. Greenhouse-Geisser correction was used if the assumption of sphericity, as checked by Mauchly's test, was violated. If a significant interaction was found between factors, post-hoc tests with Bonferroni correction were applied. To compare the Walk- DMC and muscle contributions of each synergy between groups, the independent samples t-test was used. To determine the effect sizes of tVAF, Walk-DMC and muscle weightings the partial eta squared ($\eta_p^2 \geq 0.01$, $\eta_p^2 \geq 0.06$, $\eta_p^2 \geq 0.14$) and Cohen's ($d \geq 0.2$, $d \geq 0.5$, $d \geq 0.8$) were calculated, to indicate small, moderate or large effects, respectively.

Finally, to assess the relationship between complexity of neuromuscular control and joint impairment the HJHS score was correlated with the Walk-DMC index using Pearson correlation. In addition, k-means clustering analysis was applied to identify subgroups within the PWHA (i.e. different level of joint damage in the knee and/or ankle). The groups were considered distinct if the majority of

silhouette values are larger than 0.6 (M.AqilBurney and Tariq 2014). Subsequently, the Walk-DMC was compared between the identified subgroups of PWHA with the CG-slow using one-way ANOVA and post hoc with Bonferroni correction.

RESULTS

Anthropometric and clinical characteristics

Table 1 describes participant demographic and clinical characteristics. No difference was found (p=0.541) in walking velocity of the slow condition in CG and preferred velocity in PWHA (1.0 ± 0.2 and 1.0 ± 0.2, respectively).

Neuromuscular control

The number of muscle synergies for tVAF>90% was not different between groups (i.e. median of 4 synergies, range 3-4 for CG-pref, 3-5 for CG-slow and 3-5 for PWHA), both when compared at preferred walking velocity (p=0.698) and when compared at similar velocity (p=0.540).

Comparing groups at their preferred velocity (Figure 2), two-way repeated measures ANOVA indicated a significant difference between groups (p=0.020, η_p^2=0.21) and synergy number (p<0.001, η_p^2=0.94), as well as a significant interaction (p=0.003, η_p^2=0.26). Post-hoc analysis showed a significant difference between groups only when including one synergy (p=0.003, *d*=1.32). When comparing groups for the similar walking velocity (Figure 2), two-way repeated measures ANOVA showed no significant difference between groups in tVAF as a function of the number of synergies (p=0.461, η_p^2=0.02). A significant difference between synergy number (p<0.001, η_p^2=0.95), and a significant interaction

between group and synergy number was found (p=0.007, η_p^2=0.22). Post hoc analysis showed a significant difference between groups only when including one synergy (p=0.027, *d*=0.93). These results indicate that in PWHA one muscle synergy can explain a greater part of the variance of the EMG data compared to the CG, independent of walking velocity.

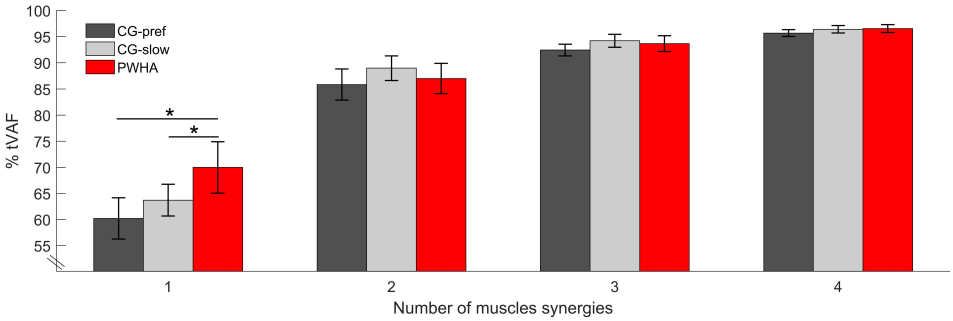


FIGURE 2 The total variance accounted for (tVAF) by one to four muscle synergies. A comparison between the control group (CG) during walking at the preferred (CG-pref) and at the slow (CG-slow) velocity and people with haemophilic arthropathy (PWHA) during walking at preferred velocity is shown. *$P < 0.05$ significant difference between CG and PWHA. Data are expressed as mean and 95% confidence intervals (n = 13 for both groups).

For the Walk-DMC index, the PWHA showed a lower value compared with CG-preferred (p=0.003, *d*=1.32) and CG-slow (p=0.027, *d*=0.92) (Fig 3). For PWHA, no significant correlation was found between HJHS (sum knee and ankle) and Walk-DMC index (r=-0.32, p=0.28).

Regarding muscle contributions of each synergy during gait at the same velocity (i.e. 1 m/s), a higher contribution was found for BF (p=0.008, *d*=1.03) and a lower for RF (p=0.023, *d*=1.21) in the acceptance synergy of PWHA (Fig. 4). During the push off synergy, a higher contribution of VL (p=0.040, d=0.90), RF (p=0.012,

d=1.10) and ST (p=0.030, *d*=0.81) was found in PWHA (Fig 4). The consequence of these results is increased co-activation between antagonistic and synergistic muscles.

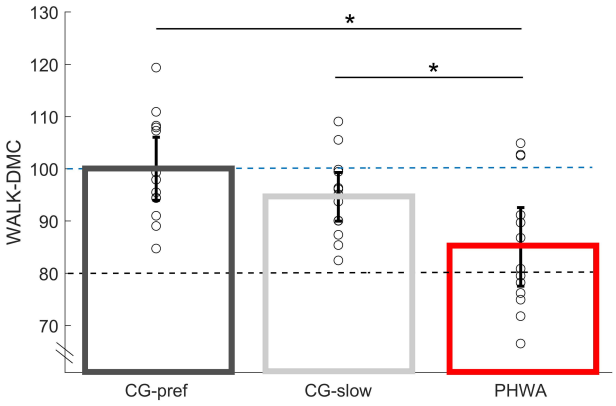


FIGURE 3 Dynamic Motor Control Index during Walking (Walk-DMC). A comparison between the control group during preferred (CG-pref) and slow walking velocity (CG-slow) and people with haemophilic arthropathy (PWHA) during preferred walking velocity. Blue horizontal dashed line indicates the normal value of Walk-DMC. Black horizontal dashed line indicates the 80 points of Walk-DMC, which is equal to two standard deviations (SD) from the normal value. *$P < 0.05$ significant difference between groups. The open circles indicate the individual data. Data are expressed as mean and 95% confidence intervals (n = 13 for both groups).

Cluster analysis of HJHS of knee and ankle and Walk-DMC index

The k-means cluster analysis for the HJHS score resulted in two clusters. The silhouette value was 0.74 ± 0.12 for cluster 1 and 0.68 ± 0.06 points for cluster 2 (Fig. 5A). Cluster 1 is characterized by a low HJHS score in the knee and cluster 2 by a high HJHS value in the knee (Figure 5B). The pain level and velocity during gait was similar between clusters (p>0.05) (Table 3).

When compared at the similar velocity, one-way ANOVA revealed a significant group effect on Walk- DMC (p=0.019, η_p^2=0.29). Post-hoc analysis indicated a significant difference only between cluster 2 and the CG (p=0.016, d=1.65) (Figure 5C). No differences between clusters (p=0.245, *d*=0.83) and between cluster 1 and the CG (p=0.921, *d*=0.41) were observed.

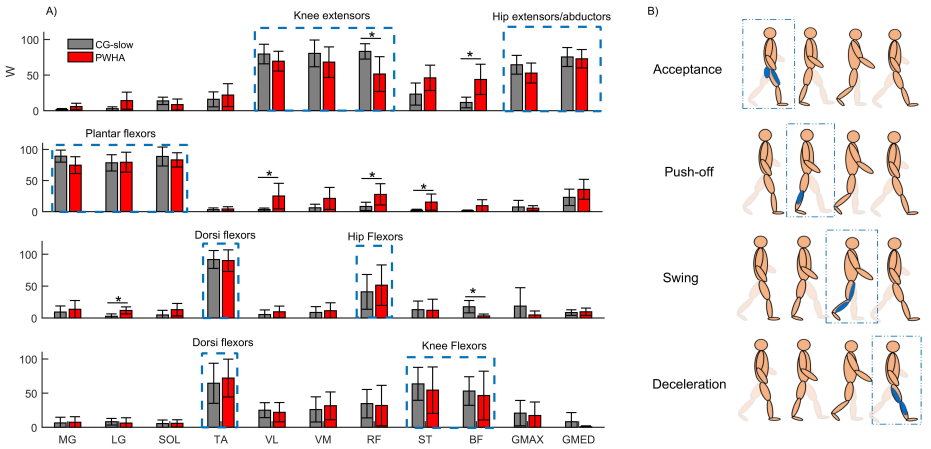


FIGURE 4 Comparisons of muscle contributions of each synergy during gait at the same velocity (ie 1 m/s). **A)** Comparisons of muscle contributions. Dashed blue line indicates the dominant muscle groups for each synergy. **B)** Schematic representation of the contribution of different muscles during gait. Dashed line and blue zone indicate the dominant muscle groups during each of the four phases. BF, Biceps Femoris; GMAX, Gluteus Maximus; GMED, Gluteus Medius; LG, Lateral Gastrocnemius; MG, Medial Gastrocnemius; RF, Rectus Femoris; SOL, Soleus; ST, Semitendinosus; TA, Tibialis Anterior; VL, Vastus Lateralis; VM, Medialis. Muscle weightings for each synergy (W). Slow walking velocity for the control group (CG-slow) and people with haemophilic arthropathy (PWHA) *P<0.05 significant difference between groups. Data are expressed as mean and 95% confidence intervals (n = 13 for both groups).

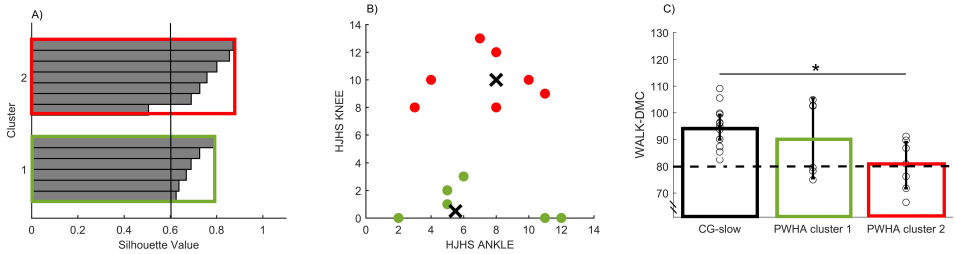


FIGURE 5 Relationship between joint impairment and Walk-DMC. **A)** The silhouette values. Note that the continuous line indicates the cut-off point (i.e 0.6 value). **B)** Haemophilia Health Joint (HJHS) scores for knee and ankle of the limb evaluated with sEMG of all individuals for each cluster (green = 1, red = 2). Cluster 1 represents the people with haemophilic arthropathy (PWHA) with lower HJHS for the knee, while cluster 2 represents those PWHA with a high HJHS value for both knee and ankle. C, Comparison of the Dynamic Motor Control Index during Walking (Walk-DMC) at the same velocity (ie 1 m/s) between the control group during slow walking velocity (CG-slow, n=13), cluster 1 (n = 6) and cluster 2 (n = 7). Black horizontal dashed line indicates the 80 points of Walk-DMC, what is equal to two standard deviations (SD) from the normal value. *P < 0.05 significant difference between groups. Data are expressed as the mean and 95% confidence intervals. The circle indicates the individuals.

TABLE 3 Clinical characteristic between clusters in PWHA.

Clinical variables	Cluster 1 (n = 6)	Cluster 2 (n = 7)	*P*-value
Evaluated limb			
HJHS ankle (points)	6.8 ± 3.8	7.3 ± 2.9	0.814
HJHS knee (points)	1.0 ± 1.2	10 ± 1.9	<0.001**
Sum HJHS knee and ankle (points)	7.8 ± 3.7	17.3 ± 3.7	<0.001**
Contralateral limb			
HJHS ankle (points)	3.7 ± 4.5	6.6 ± 5.0	0.300
HJHS knee (points)	1.2 ± 1.3	6.1 ± 6.0	0.077
Sum HJHS knee and ankle (points)	4.8 ± 4.2	12.7 ± 10.5	0.113
Pain and walking			
Pain during walking (VAS 0-10)	0 (0.5)	0 (0.6)	0.334
Preferred velocity in 30 m (m/s)	1.10 ± 0.19	0.99 ± 0.14	0.238
Physical activity (>150min/wk)	1/6	3/7	0.308

Parametric distribution: Mean ± SD. Nonparametric distribution: Median [Range]. HJHS, Haemophilia Joint Health Score; VAS, Visual Analogue Scale. **P-value <0.001 for comparison between clusters

DISCUSSION

The main results of this study are: (1) that on average the complexity of neuromuscular control (i.e. the extent of muscle co-activation) during gait in PWHA was different from healthy controls; and (2) that the complexity of neuromuscular control (Walk-DMC) was not correlated with ankle and knee joint health status (HJHS). To the authors' current knowledge, this study is the first to suggest an increased co- activation between synergistic and antagonistic muscles during gait in PWHA. This is clinically relevant, because a greater co-activation between antagonistic muscles could result in more intraarticular loading, resulting in greater progression of the osteoarthritis (Hodges et al. 2016).

The number of muscle synergies for tVAF > 90% in PHWA was not different than that in the CG. This is in agreement with previous reports on effects of musculoskeletal diseases (Allison et al. 2018; Feeney et al. 2018; Serrancolí et al. 2016). In contrast, several studies have reported a lower number of muscle synergies in neurological diseases (Clark et al. 2010; Steele et al. 2015). As the number of synergies is not a very distinctive measure, we also assessed other metrics of the complexity of neuromuscular control. We found significant differences between PWHA and CG for tVAF1 and Walk-DMC. For tVAF1, the mean difference between PWHA and CG was more than 7%. This difference between groups is higher than inter-day measurement error of the tVAF1 reported for patients with cerebral palsy (i.e 5%)(Shuman et al. 2016). At the individual level, there was quite some overlap of Walk-DMC values between the two clusters for PWHA and CG. This suggests that some PWHA have normal neuromuscular control of gait.

The higher tVAF1 and lower Walk-DMC in PHWA can be explained by more co-activation of synergists and antagonists (see Figure 1). This is supported the increased co-activation of knee flexors and extensors during acceptance and push-off synergies (see Figure 4). Both results have been interpreted as indications of more simplified control by the CNS during gait (Clark et al. 2010; Steele et al. 2015).

The z-score normalization of tVAF1 (i.e Walk-DMC) has some advantages compared to the tVAF1. It is affected less by the different methods of EMG processing and it can be better compared across studies (Shuman et al. 2018).

The observed changes in neuromuscular control may be explained by different mechanisms. (a) Adaptations of the CNS to pain. Considering the temporal distinction between acute and chronic pain, different sources of pain, such intra-articular bleeding, inflammation of synovium and joint degeneration, as well alteration in pain perception in PWHA (e.g altered central pain mechanisms) may change the kinematics and kinetics of gait (Hodges 2011; Roussel 2018). However, the pain levels during walking in the PWHA of the present study were rather low (see Table 1 and 2). Therefore, we do not expect pain to play an important role in our results. (b) The reduced range of ankle and knee joint motion. The chronic limitation of range of motion and disuse of the affected limb in PWHA may affect the mechanical properties of muscle and tendon (Cruz-Montecinos et al. 2019b). Previous studies reported moderate-to-high correlations between muscle weakness and tVAF1 in patients with cerebral palsy and Duchenne muscular dystrophy (Goudriaan et al. 2018). In the present study, we did not find a significant correlation between the total HJHS score and Walk-DMC. However, this may be related to the limitations of the HJHS score. The HJHS involves a qualitative assessment with a small resolution (i.e few levels), manual testing of force and

testing range of motion during passive conditions only. We propose to include quantitative assessments of muscle strength and measurements of joint range of motion during gait in future studies. On the other hand, the subgroup of PWHA with an impairment of both knee and ankle joints showed a decrease in complexity of neuromuscular control (Walk-DMC) during gait compared to the subgroup with limitations in only the ankle joint. This indicates that neuromuscular control of gait is affected more in multi- joint impairment. (c) Disrupted proprioception. In PWHA, alterations in proprioceptive performance have been reported (i.e angle-reproduction test) (Hilberg et al. 2001). (d) Impaired postural control. It has also been shown that static balance control is affected in PWHA (Cruz-Montecinos et al. 2017; Gallach et al. 2008). The enhanced co-activation between flexors and extensors during gait, increasing joint stiffness, could be related to the reduced proprioception and impaired postural control (Smith et al. 2019; Thompson et al. 2018).

In the clinical context, the tVAF1 and Walk-DMC may be of additive value to assess the quality of neuromuscular control. Different approaches have been reported to enhance muscle function and joint range of motion, and reduce pain in PWHA (Schäfer et al. 2016). However, in PWHA little is known about the impact of physical therapy on neuromuscular control. The selection of the best exercise protocol to reduce the extent of co-activation between joint flexor and extensor muscles could be relevant to prevent joint deterioration (Hodges 2011; Hodges et al. 2016; Smith et al. 2019). This may help to improve solutions for rehabilitation after conservative treatment and orthopaedic surgery (Escobar et al. 2018; Schäfer et al. 2016).

A limitation of this study is that joint kinematics and kinetics were not assessed. Therefore, it is unclear if the gait movement pat-tern and mechanics were different in PWHA, and if this was related to the changes in neuromuscular control. In addition, a thorough analysis of the relationship between joint structure, muscle dysfunction and neuromuscular control is warranted, by adding radiological exams such as computed tomography, magnetic resonance imaging and sonography.

CONCLUSION

The complexity of neuromuscular control during gait was reduced in PWHA by means of increased co-activation between synergistic and antagonistic muscles. Our results indicate that neuromuscular control is affected more in PWHA with multi-joint damage com-pared to single joint damage. The tVAF1 and Walk-DMC may be useful measures to assess changes in neuromuscular control in PWHA before and after rehabilitation therapies and or orthopaedic surgical interventions.

CHAPTER 3

Altered neural control of gait and its association with pain and joint impairment in adults with haemophilic arthropathy: Clinical and methodological implications

Introduction: It is unknown whether altered neural control is associated with clinical outcomes in people with haemophilic arthropathy (PWHA). The dynamic motor control index during walking (Walk-DMC) is a summary metric of neural control. **Aims:** The primary aim of this study was to apply the Walk-DMC to assess if people diagnosed with haemophilic arthropathy have impaired neural control of gait and investigate the association of Walk-DMC with pain and joint impairment. **Method:** The Walk-DMC was assessed using surface electromyography in 11 leg muscles. Twenty-two PWHA and 15 healthy subjects walked on a 30-m walkway at 1 m/s. In addition, pain (visual analogue scale), knee flexion contracture (degrees) and joint impairment (Haemophilia Joint Health Score, HJHS) were assessed. The clinical outcomes were correlated with the Walk-DMC. Multiple regression analysis was per-formed to predict the Walk-DMC using the clinical outcomes. **Results:** In 13 PWHA the Walk-DMC was beyond the normal range (80–120 pts). PWHA with an altered Walk-DMC showed more years with arthropathy, more pain, higher knee flexion contracture and a higher HJHS score ($P < .05$, effect size > .8). Significant negative moderate associations between Walk-DMC and pain, knee

flexion contracture and HJHS were found ($P < .05$). The model that best predicted the Walk-DMC was the pain with knee flexion contracture ($R^2 = .44$; $P = .004$). **Conclusions:** PWHA with abnormal neural control of gait also has more years with arthropathy, more pain, and more impaired joints. Our results indicate an association between the Walk-DMC index and joint damage, specifically with pain in combination with knee flexion contracture.

Cruz-Montecinos C, Maas H, Cerda M, Pérez-Alenda S. Altered neural control of gait and its association with pain and joint impairment in adults with haemophilic arthropathy: Clinical and methodological implications. Haemophilia. 2022. *doi: 10.1111/hae.14517.*

INTRODUCTION

In people with haemophilia, the most frequent clinical manifestation is haemophilic arthropathy, which results from repetitive intraarticular bleeding, inflamed synovial membrane and irreversible changes in cartilage tissue (van Vulpen et al. 2018). Prophylaxis is accepted as the only way to prevent bleeding and preserve musculoskeletal health (Srivastava et al. 2020). Without adequate prophylaxis treatment, the process of joint deterioration in haemophilic arthropathy can be seen with an aggressive progression rate. However, in many underdeveloped countries, prophylaxis availability is limited (Boadas et al. 2018).

Haemophilic arthropathy often results in chronic pain and joint impairment, affecting motor function and quality of life (Krüger and Hilberg 2020; Lobet et al. 2010, 2021; McLaughlin et al. 2017; Stephensen et al. 2012; van Vulpen et al. 2018). Clinical examination of joints and muscles, besides examining posture and gait, are the most often used assessments by physiotherapists for haemophilia care (Stephensen et al. 2019). In addition, several tools have been proposed to monitor disease progression, such as ultrasound and magnetic resonance imaging of joints, gait analysis and surface electromyography (sEMG) (Lobet et al. 2020; Pasta et al. 2020; Putz et al. 2020; Seuser et al. 2018; Stephensen et al. 2016). Using sEMG, a new tool called the Walking Dynamic Motor Control (Walk-DMC) index has been proposed as a summary metric of neural control of gait. The Walk-DMC index has been used to assess synergistic and antagonistic co-activation changes in response to neurological diseases, following orthopaedic surgeries, in the elderly and in people with haemophilic arthropathy (PWHA) (Collimore et al. 2021; Cruz-Montecinos et al. 2019a; Schwartz et al. 2016; Steele et al. 2015). However,

whether PWHA and an abnormal Walk-DMC also have different levels of pain and joint impairment than those with a normal Walk-DMC and whether the Walk-DMC is associated with the clinical measures of pain and joint impairment are currently unknown.

Assessment of the Walk-DMC index is based on a muscle synergy analysis of gait (Schwartz et al. 2016; Steele et al. 2015). This analysis assumes that muscles are activated in groups, commonly referred to as synergies or modes (Ivanenko et al. 2004; Tresch et al. 1999). Using synergy analysis, we recently found that some synergies were merged in PWHA with a chronic knee constraint (Cruz-Montecinos et al. 2021). In comparison to complete synergy analysis (i.e. number of synergies, variance accounted for, motor modules and motor primitives), the Walk-DMC index is a metric that can be interpreted rapidly (Collimore et al. 2021; Cruz-Montecinos et al. 2019a; Schwartz et al. 2016; Shuman et al. 2016; Steele et al. 2015). Hence, it may be applied clinically, for example to evaluate the effects of orthopaedic interventions (Schwartz et al. 2016). The Walk-DMC has been assessed using the sEMG signals of 5–11 muscles (Bekius et al. 2020; Collimore et al. 2021; Cruz-Montecinos et al. 2019a; Schwartz et al. 2016; Steele et al. 2015).[14–17,22] However, it is unknown if the number of muscles selected affects this metric.

The primary aim of this study was to apply the Walk-DMC to assess if people diagnosed with haemophilic arthropathy have impaired neural control of gait and investigate the association of the Walk-DMC with pain and joint impairment. The secondary aims were to assess possible clinical predictors of the Walk-DMC and determine the minimal number of muscles required to detect an altered Walk-DMC in PWHA accurately.

MATERIAL AND METHODS

Participants

This study was approved by the local ethics committee and conducted in agreement with the Declaration of Helsinki. Part of the data used has been reported in a previous study, which addressed a different research question related to neuromuscular control during gait in PWHA (Cruz-Montecinos et al. 2019a). All participants were informed about the purpose and procedures of the project and gave their written informed consent to participate in the study. Based on non- probability sampling, PWHA were recruited in two hospitals in Santiago (Chile). For the control group (CG), healthy subjects were recruited from the University of Chile (student and employees). The inclusion criteria for PWHA were males, diagnosed with haemophilia A or B, haemophilic arthropathy with a minimum of two points (sum knee and ankle in the evaluated leg) by the Haemophilia Joint Health Score (HJHS)(Hilliard et al. 2006; Sun et al. 2014), over 18 and under 65 years of age, prophylaxis treatment with the deficient factor (i.e. VIII or IX) and body mass index lower than 35 kg/m2 with the aim of decreasing the potential effect of subcutaneous fat tissue that can reduce the sEMG amplitude by working as a low-pass filter (Cooper et al. 2014). The exclusion criteria were history of hip, knee or ankle arthroplasty in the evaluated leg; equinus foot; inability to walk without an assistive device; history of muscle or joint bleeding in the lower limbs in the last two months; chronic cardiac and/or respiratory pathology and neuro-logical disease.

For CG, the inclusion criteria were the following: male, over 18 and under 65 years of age and body mass index lower than 35 kg/m^2. The exclusion criteria were the

following: acute traumatic injuries or chronic musculoskeletal disorders, signs or symptoms of injury or symptomatic arthritis to the trunk, lower back and lower limb within the past three months, any single positive findings of the Altman's criteria for knee osteoarthritis (Altman et al. 1986), history of musculoskeletal surgery in the lower limb and spine, scoliosis; bleeding disorders, cardiac and/or respiratory pathology and neurological disease.

Twenty-two people with severe (n = 19) and moderate (n = 3) haemophilia were recruited (32.3 ± 11.6 years, body mass index 25.6 ± 3.7 kg/m2, a total HJHS of 41.0 ± 20.4 pts). To calculate the Walk-DMC (see below), 15 healthy control subjects were recruited (31.5 ± 10.1 years, body mass index 24.5 ± 1.9 kg/m2). Both groups showed similar age and anthropometric characteristics (P > .05).

Clinical assessment in people with haemophilic arthropathy

The Visual Analog Scale (VAS 0–10 pts) was used to assess pain intensity during barefoot walking. For each participant, the HJHS 2.1 was used to assess joint impairment (Hilliard et al. 2006; Sun et al. 2014). The HJHS score was applied to the knee and ankle (0–40 pts) of the limb assessed. Gait impairment was evaluated using the global gait score (0–4 pts) of the HJHS. In addition, the total score on the HJHS (0–124 points) was included. At the joint level, the HJHS has a good correlation with X-rays (Pettersson score), and the total HJHS has a moderate correlation with self-reported functions (Poonnoose et al. 2016; Ribeiro et al. 2021). Furthermore, the knee flexion contracture (i.e. loss of joint extension) was assessed to measure knee joint deformity. For setting the knee contracture angle and HJHS, a universal goniometer with a 1° increment (Baseline®, Chattanooga Group Inc) was used.

Data acquisition

In PWHA, the limb with the highest score on the HJHS was selected for the experiment. In the CG, the dominant limb, which was determined by asking the subjects which leg they would use to kick a ball, was assessed (Cruz-Montecinos et al. 2019a). After shaving and cleaning the skin with alcohol, sur-face bipolar electrodes of 2.4 cm diameter (Ag–AgCl, Kendall H124SG) were placed such that the interelectrode spacing was 2 cm on the leg muscles. The localisation of electrodes in the leg muscles (see Table 1) was made according to SENIAM guidelines (Hermens et al. 2000). Muscle activity patterns were assessed using a wireless sEMG system (MyoSystem DTS; Noraxon USA Inc, Scottsdale, California, USA), with a sampling rate of 1500 Hz. Gait cycle events were detected by a synchronised wireless pressure sensor placed underneath the heel of the foot.

Experimental protocol

All PWHA walked barefoot twice for 30 m, with 2 minutes of rest in between tests. PWHA were invited to walk at their preferred velocity. For each participant, the time elapsed in each 30 m walk test was used as a marker of the walking velocity. From 1 to 2 hours before the experiment, PWHA received prophylactic treatment. The CG walked barefoot overground for 30 m twice at a slower velocity to reproduce the mean preferred velocity of PWHA (i.e. 1.0 m/s). To ensure that the walking speed was performed correctly, the time spent travelling 30 m was registered, and immediate feedback on velocity was given to each participant. It is critical to matching the walking velocity between CG and the target population so as not to affect the interpretation of neural control during gait (Shuman et al. 2016).

Walk-DMC calculation

For each group, 20 gait cycles were included in the analysis (Cruz-Montecinos et al. 2019a; Shuman et al. 2016). The Walk-DMC was calculated based on previous studies on neurological diseases and PPWHA 14–16 For the details of sEMG signal processing and Walk-DMC calculation, see the complementary material. Briefly, the Walk-DMC was calculated for each PWHA using the z-score normalisation, including the mean and standard deviation of total variance accounted for one synergy ($TVAF_1$) from the CG at a fixed walking velocity (1 m/s)(Schwartz et al. 2016; Steele et al. 2015). $TVAF_1$ has been shown to be repeatable between days in healthy children and those with cerebral palsy (Shuman et al. 2016; Steele et al. 2019). In addition, assessment of Walk-DMC has been reported to be consistent between different motion laboratories for both children and young adults (ages 4–21) (MacWilliams et al. 2021). Seven selections of muscles (from 5 to 11 leg flexors and extensors) were used to calculate the Walk-DMC. The 11 hip, knee and ankle flexors and extensors were selected based on the four muscle synergies described during walking: acceptance synergy (hip and knee extensors), propulsion synergy (ankle extensors), swig synergy (ankle dorsiflexors and hip flexors) and deceleration synergy (knee flexors) (Ivanenko et al. 2004).The five muscles used in all selections were based on a previous paper (Steele et al. 2015). Subsequently, muscles were added randomly one by one until all included muscles were selected (see Table 1). Note that the configuration with five muscles includes a representation of the four synergies with the representation of at least one muscle (acceptance, rectus femoris; propulsion, medial gastrocnemius; swing, tibialis anterior and rectus femoris; deceleration, semitendinosus and bicep femoris) (Ivanenko et al. 2004). A Walk-DMC of 100 points is equal to the neural control of CG. An index between 80 and 120 (i.e. + and – two standard deviations from the mean of the CG) was considered within the normal range (Schwartz et al. 2016; Steele et al. 2015).

TABLE 1 Muscles configurations used to calculate the Walk-DMC index

Five	Six	Seven	Eight	Nine	Ten	Eleven
MG	MG	MG	MG	MG	MG	MG
TA	TA	TA	TA	TA	TA	TA
RF	RF	RF	RF	RF	RF	RF
BF	BF	BF	BF	BF	BF	BF
ST	ST	ST	ST	ST	ST	ST
	VM	VM	VM	VM	VM	VM
		VL	VL	VL	VL	VL
			LG	LG	LG	LG
				GMAX	GMAX	GMAX
					SOL	SOL
						GMED

Abbreviations: MG, medial gastrocnemius; TA, tibialis anterior; RF, rectus femoris; BF, biceps femoris; ST, semitendinosus; VM, vastus medialis; VL, vastus lateralis; LG, lateral gastrocnemius; GMAX, Gluteus maximus; SOL, soleus, GMED, gluteus medius.

Statistical analysis

The sample size was calculated considering the negative association reported between plantar flexor strength and tVAF1 in patients with cerebral palsy (r =−.72) (Goudriaan et al. 2018). Twenty-two PWHA were determined to be sufficient to reach a P value of .05 and β of .20. The normality of the data was evaluated through the Shapiro-Wilk test. P values smaller than .05 were considered statistically significant.

For the primary aim, the clinical characteristics between PWHA with a normal Walk-DMC and those with an altered one were compared using the 11 hip, knee and ankle flexor and extensor muscles (gold standard). To compare the clinical characteristics between normal and altered Walk-DMC, the independent t-test (if

data were normally distributed) or Wilcoxon rank- sum test (if data were not normally distributed) was used. The effect size was calculated based on the t-value (normal distributed) or z-value (not normal distributed). All effect sizes were transformed to *Cohen's d*. *Cohen's d* has been operationally described in the following ranges: < .2 (no effect), .2–.5 (small effect), .5–.8 (moderate effect) and > .8 (large effect). Furthermore, the clinical outcomes (i.e. pain, knee flexion contracture and total HJHS of the limb) were correlated with the Walk-DMC index. The Pearson (normal distributed) and Spearman (not nor-mal distributed) analyses were used. The correlation coefficient (*r*) was interpreted as < .39 (week association), .4–.60 (moderate association) and .60–1 (strong association). For the secondary aim, multiple regression was applied to assess the predictor variables of the Walk-DMC index. The different variables (VAS, knee flexion contracture and HJHS of the limb) were entered conditionally using the forward method. Furthermore, the agreement between the 5–10 selected muscles and the gold standard (11 muscles) to detect the altered Walk-DMC was calculated using the Kappa statistic. The kappa was interpreted as none (≤ 0), no or weak agreement (.01–.20), fair (.21–.40), moderate (.41–.60), substantial (.61–.80) and almost perfect (.81–1.00).

RESULTS

Differences in clinical characteristics between normal and altered neural control

Neural control was found to be altered (i.e. Walk-DMC < 80) in 13 out of 22 PWHA. PWHA with an altered Walk-DMC showed more years with arthropathy, more pain, higher knee flexion contracture and a higher HJHS score (Table 2, Figure1A) than those with a normal Walk-DMC index. We also found that PHWA

with an altered Walk-DMC index showed a tendency to start prophylactic treatment later than those with a normal Walk-DMC index (P = .181, moderate effect) (Table 2). The colour map of muscle activation during gait indicates that PWHA with altered neural control have greater co-activation of hip, knee and ankle joint muscles, particularly at the beginning of the stance phase (Figure 1B).

TABLE 2 Clinical characteristics of normal and altered Walk-DMC index using 11 muscles

Clinical variables	Normal index (n=9)	Altered index (n=13)	p-value	Effect size
Age	26 [20 42]	33 [22 64]	*.047	.9 (large)
Pain (VAS 0–10)	1 [0 4]	4 [0 8]	*.021	2.9 (large)
Chronicity of arthropathy (years)	7 [3 12]	13 [5 35]	*.010	1.3 (large)
Start of prophylaxis (age)	16 [2 38]	26 [12 61]	.181	.6 (moderate)
Knee flexion contracture (degrees)	5 [0 20]	20 [0 35]	*.012	1.2 (large)
HJHS limb (0-40)	12.0 ± 5.8	19.9 ± 6.0	*.006	1.0 (large)
HJHS global gait (0-4)	3 [0 4]	4 [1 4]	*.043	.9 (large)
Total HJHS (0-124)	27.0 ± 17.5	50.8 ± 16.6	*.004	1.4 (large)
Walking velocity (m/s)	1.1 ± .1	1.0 ± .2	.162	.6 (moderate)

Visual analogue scale (VAS). Haemophilia joint health score (HJHS). Normal distribution: Mean ± SD. Not normal distribution: Median [Range]. *$P < .05$.

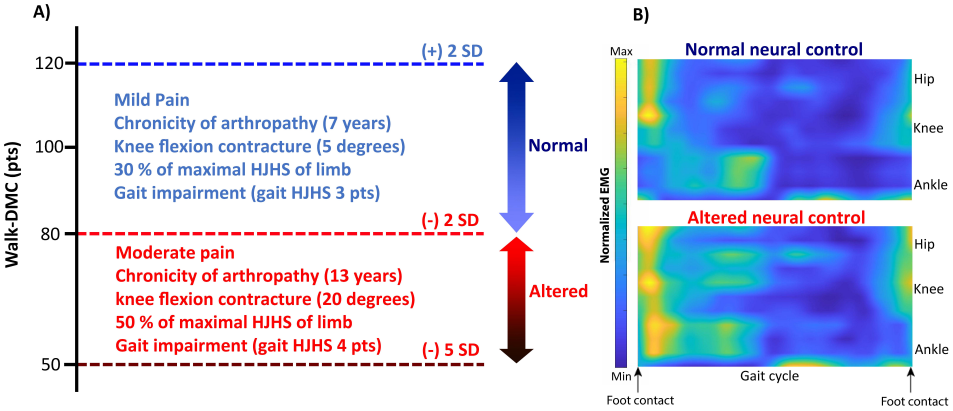


FIGURE 1 (A) Clinical interpretation of those people with haemophilic arthropathy with and without altered Walk-DMC. Haemophilia health joint score (HJHS). Gait impairment was defined as the maximum score of the gait score of the HJHS (i.e. 4 points). (B) Colour map of the average muscle activation pattern of PWHA with normal and altered neural control of gait. The gait cycle starts with the stance phase. Muscle activity of each muscle in each participant was normalized to the maximum value of all included cycles (20 cycles). The yellow colour represents the maximum value (1) of the normalized muscle activity. Electromyography (EMG).

Association between altered Walk-DMC and clinical outcome measures

Significant, negative, moderate associations between Walk-DMC and pain (r =−.59, P = .004), knee flexion contracture (r =−.58, P = .004) and HJHS of the limb (r =−.52, P = .013) were found (Figure 2).

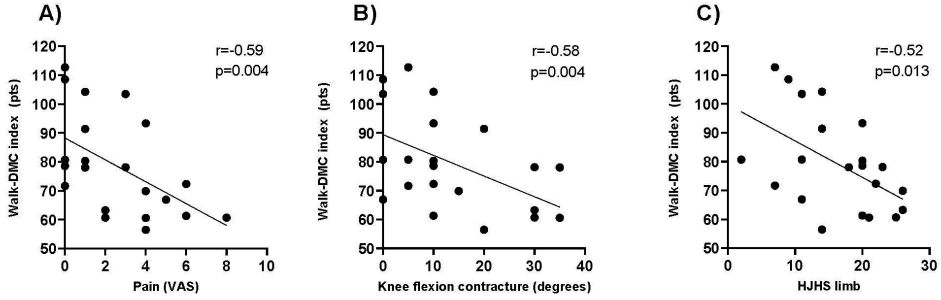


FIGURE 2. Correlations between Walk-DMC and clinical outcomes. Haemophilia health joint score (HJHS). Visual analogue scale (VAS).

Predictors of the Walk-DMC index

The regression analysis with the forwarding method includes two models (model A: pain and model B: pain with knee flexion contracture) (Table 3). The model that best predicted the Walk-DMC index was the pain with knee flexion contracture, which explained 44% of the variance of the Walk-DMC with a standard error of 13 points (Table 3). The association between pain and knee flexion contracture was not significant (r = .35, P = .107). The variance inflation factor was 1.07 for both variables, indicating an absence of multicollinearity. The residuals were normally distributed.

The number of muscles to detect the altered Walk-DMC

The number of muscles included affected the value of the Walk-DMC index and thereby the number of subjects with an abnormal value (Figure 3A and 3B). For a substantial agreement (kappa > .6) with the gold standard (i.e. 11 muscles), a minimum of eight knee and ankle flexors and extensors were needed (Figure 3C).

DISCUSSION

The main results of this study are: (i) The neural control of gait was not altered in all PWHA; (ii) PWHA with an altered Walk-DMC showed more years with arthropathy, experienced more pain and had a higher knee flexion contracture and greater joint and gait impairment; (iii) The Walk-DMC was moderately associated with pain, knee flexion contracture and HJHS; (iv) Pain level and knee flexion contracture explained 44% of the variance of the Walk-DMC index; (v) A minimum of eight knee and ankle flexor and extensor muscles need to be included in the assessment of Walk-DMC. To the authors' current knowledge, this is the first study

that reports the association of pain and joint impairment with altered neural control of gait in PWHA.

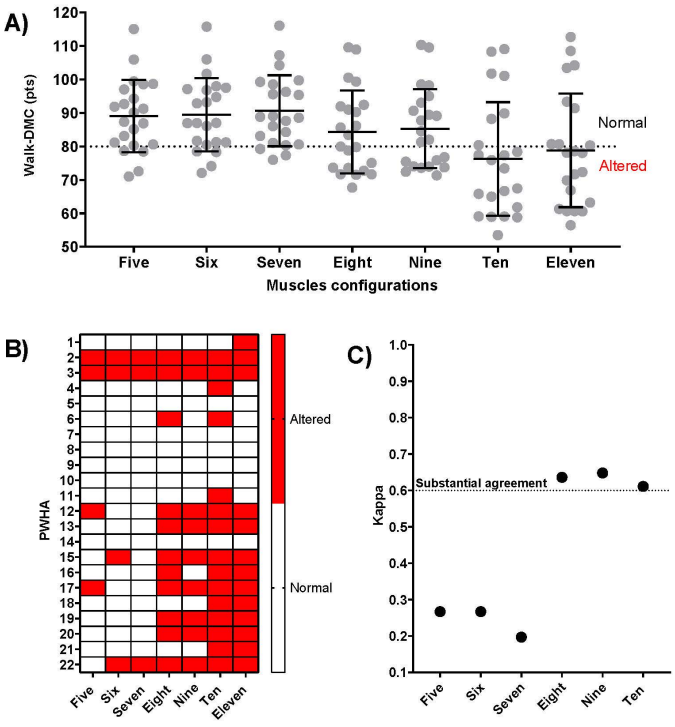


FIGURE 3 (A) The Walk-DMC index in people with haemophilic arthropathy for the different muscle configurations. (B) Detection of altered Walk-DMC index in people with haemophilic arthropathy for the different muscle configurations. (C) The agreement between the different configurations (5–10 muscles) and the gold standard configuration (11 muscles), as assessed with the kappa statistical test.

Clinical interpretation of altered neural control of gait in PWHA

PWHA with altered neural control of gait experienced more pain and 50% of the maximal score of HJHS of the limb. The association between neural control and pain in PWHA has not been reported before. Previously, our research group reported

that neural control is affected in some PWHA, but no significant association with joint damage was found (Cruz-Montecinos et al. 2019a). The small sample size and lower joint damage amongst the recruited PHWA are potential reasons for the absence of a significant association (Cruz-Montecinos et al. 2019a).In children with cerebral palsy and Duchenne muscular dystrophy, a moderate-to-high association between limb muscle weakness and $TVAF_1$ has been reported (Goudriaan et al. 2018). In people with knee osteoarthritis, increased co-activation between antagonists during gait has been associated with disease severity (Mills et al. 2013a), but this was not based on the Walk-DMC. Pain and joint impairment may affect neural control of gait by different mechanisms. First, pain may cause increased co-activation between antagonists as a strategy to protect tissues from further injury (Hodges 2011; Mills et al. 2013a). Second, joint deterioration can be accompanied by altered proprioception affecting joint and gait stability, which may be compensated by increased co-activation of antagonists (Deschamps et al. 2018; Hilberg et al. 2001; Mills et al. 2013a).

We also observed that PWHA with altered neural control of gait had more significant knee flexion contracture, more years with arthropathy, and a tendency to start prophylaxis treatment later than those with a normal Walk-DMC (see Table 2). The mechanical constraints on the knee may explain the altered neural control in PWHA. How-ever, in healthy individuals, a simulated severe knee flexion contracture (20°) did not change the neural control of gait (Cruz-Montecinos et al. 2021). Only long-term expo-sure to a knee joint constraint of a substantial amplitude (≈ 20°) seems to cause structural changes in the neural control of gait (Cruz-Montecinos et al. 2019a). Mechanically, a knee flexion contracture may result in higher loads on a smaller joint surface area, which may increase the level of pain, alter the neural control of gait and increase joint stiffness (Campbell and McGonagle 2021; Cruz-Montecinos et al. 2021). Long-term exposure to arthropathy, pain and

a joint constraint may cause secondary adaptations of the central nervous system (Cruz-Montecinos et al. 2021). Once such adaptations have occurred, it may be challenging to return neural control to normal (e.g. after total knee arthroplasty).

It is important to note that some individuals with abnormal Walk-DMC index values showed low pain and knee flexion contracture (Figure 2), indicating the variability of neural control of gait in response to arthropathy. To understand the potential inter-relationship between clinical outcomes, we performed a multiple regression analysis to predict the Walk-DMC index using the clinical outcomes (pain, knee flexion contracture and HJHS of the limb). We observed that pain with knee flexion contracture was the model that best predicted the Walk-DMC index (Table 3). However, other non-mechanical factors such altered pain processing, kinesiophobia and catastrophism may also affect neural control and should be addressed in future studies (Krüger and Hilberg 2020; De Oliveira Silva et al. 2020).

Minimal number of muscles for valid assessment of the Walk-DMC

It has been suggested that the number of muscles recorded appears to influence the outcomes of synergy calculations (Bekius et al. 2020; Steele et al. 2013). We found that the Walk-DMC based on the sEMG signal from five to eight muscles was different from that based on 11 muscles. Our results agree with the study by Steele et al. (2013), which reported that a small number of muscle configurations might underestimate the complexity of neuro-muscular control. We propose that the sEMG of a minimum of eight leg muscles needs to be included in assessment of the Walk-DMC.

Limitations

To determine the minimal number of muscles required for a valid assessment of the Walk-DMC in PWHA, we tested only a random selection of 5–11 muscles. Another approach, such as muscle selection by size (i.e. volume and cross-sectional area), may help to under-stand motor impairment related to altered Walk-DMC index. More-over, proprioception was not assessed. Therefore, it was not possible to associate the changes in somatosensory processing and the alteration of neural control of gait. Finally, although the Walk-DMC is repeat-able between days and consistent between motion laboratories (MacWilliams et al. 2021; Shuman et al. 2016; Steele et al. 2019), reproducibility data are not available for PWHA.

CONCLUSION

PWHA with abnormal neural control of gait also have more years with arthropathy, more pain and more impaired joints. Our results indicate an association between the Walk-DMC index and joint damage, specifically with pain in combination with knee flexion contracture. However, the assessment of the Walk-DMC index is sensitive to the number of muscles used for sEMG measurements. The Walk-DMC index may be used as an additional assessment tool to monitor disease progression in PWHA.

Complementary material

Signal processing and Walk-DMC calculation.

The Matlab software 2019a was used for all (MathWorks, Inc, Natick, MA, USA). A bandpass filter (20-500 Hz) followed by rectification using Hilbert transformation was applied. Subsequently, sEMG signals were low pass filtered at 10 Hz and normalized to the maximum value of all included cycles (20 cycles).

A more detailed description can be found in a previous paper (Cruz-Montecinos et al. 2019a; Schwartz et al. 2016). Non-negative matrix factorization (NNMF) was used to extract muscle synergies from the EMG signals. The difference between the reconstruction of sEMG data from the composition and timing of one synergy, and the original sEMG data was calculated to obtain the total variance accounted for one synergy (TVAF1). The sEMG data was time normalized to 200 points. The NNMF was used to extract muscle synergies from the EMG signals. The total number of allowable synergies was constrained to one. The sEMG were combined into an m × t matrix, where m represents the number of muscles (5-11) and cycles (20 cycles), and t is the time base (200 points). The NNMF algorithm was iterated 20 times, and the iteration with the lowest reconstruction error was selected. The difference between the reconstruction of sEMG data from the composition and timing of one synergy, and the original sEMG data was calculated to obtain the $TVAF_1$.

The Walk-DMC was calculated for each PWHA using the z-score normalization, including the mean and standard deviation (SD) of $TVAF_1$ from the CG at a fixed walking velocity (1 m/s) (see formula 1).

1)

$$\boldsymbol{Walk\ DMC\ index = 100 + 10\left[\frac{\text{MEAN TVAF}_1\text{(CG)} - \text{TVAFT}_1\ \text{(individual)}}{SD\ TVAF_1\ \text{(CG)}}\right]}$$

CHAPTER 4

Changes in Muscle Activity Patterns and Joint Kinematics During Gait in Haemophilic Arthropathy

Introduction: Haemophilic arthropathy is the result of repetitive intra-articular bleeding and synovial inflammation. In people with haemophilic arthropathy (PWHA), very little is known about the neural control of individual muscles during movement. **Aims:** The aim of the present study was to assess if the neural control of individual muscles and coordination between antagonistic muscle pairs and joint kinematics during gait are affected in PWHA. **Methods:** Thirteen control subjects (CG) walked overground at their preferred and slow velocity (1 m/s), and 14 PWHA walked overground at the preferred velocity (1 m/s). Joint kinematics and temporal gait parameters were assessed using four inertial sensors. Surface electromyography (EMG) was collected from gluteus maximus (GMAX), gluteus medius (GMED), vastus medialis (VM), vastus lateralis (VL), rectus femoris (RF), medial gastrocnemius (MG), lateral gastrocnemius (LG), soleus (SOL), tibialis anterior (TA), semitendinosus (ST), and biceps femoris (BF). Waveforms were compared using the time-series analysis through statistical parametric mapping. **Results:** In PWHA compared to CG, EMG amplitude during the stance phase was higher for LG (for both velocities of the CG), BF (slow velocity only), and ST (preferred velocity only) ($p < 0.05$). Co-contraction during the stance phase was higher for MG-TA, LG-TA, VL-BF, VM-ST, LG-VL, and MG-VM (both velocities) ($p < 0.05$). MG and LG were excited earlier (preferred velocity only) ($p < 0.05$). A later offset during the

stance phase was found for VL, BF, and ST (both velocities), and BF and GMAX (preferred velocity only) ($p < 0.05$). In addition, the range of motion in knee and ankle joints was lower in PWHA (both velocities) and hip joint (preferred velocity only) ($p < 0.05$). **Conclusions:** In conclusion, the neural control of individual muscles and coordination between antagonistic muscles during gait in PWHA differs substantially from control subjects.

Cruz-Montecinos C, Pérez-Alenda S, Querol F, Cerda M, Maas H. Changes in Muscle Activity Patterns and Joint Kinematics During Gait in Hemophilic Arthropathy. Front Physiol. 2020. 31; 10:1575. *doi: 10.3389/fphys.2019.01575.*

INTRODUCTION

Haemophilia is an X chromosome-linked bleeding disorder caused by a deficiency of coagulation factors VIII (haemophilia A) and factor IX (haemophilia B) (Oldenburg et al. 2004). The prevalence of haemophilia A (12.8 per 100,000 male) is higher than that of haemophilia B (1.6 per 100,000 male) (Stonebraker et al. 2012). The most common clinical manifestation of haemophilia is arthropathy, affecting 90% of people with severe haemophilia (Manco-Johnson et al. 2004). Haemophilic arthropathy is the result of repetitive intra-articular bleeding and synovial inflammation, characterized by joint impairment, chronic pain, and reduced quality of life (Fischer et al. 2005, 2016; Krüger et al. 2018; Roussel 2018). The intra-articular bleeding and the inflamed synovium generate an irreversible change in cartilage tissue (Pulles et al. 2017). This is mediated by chondrocyte apoptosis, resulting in the inability of chondrocytes to restore proteoglycan synthesis, eventually leading to joint destruction (Hooiveld et al. 2003). The synovial hypertrophy and hypervascularization increase the sensitivity for bleedings during tasks involving low loads on the joints (Melchiorre et al. 2017; Pulles et al. 2017). Animal studies have reported that a single hemarthrosis results in irreversible damage of cartilage (Hakobyan et al. 2008; Hooiveld et al. 2003; Madhok et al. 1988; Roosendaal et al. 1997), indicating that joint impairment in people with haemophilic arthropathy (PWHA) could be observed following a single hemarthrosis event.

In the lower limb of PWHA, the knee and ankle joints are the most commonly affected. The joint damage is accompanied by changes in the properties of the neuromusculoskeletal system, as a reduced passive range of joint motion (Soucie et al. 2004), muscle size (Stephensen et al. 2012), maximal muscle force (González

et al. 2007; Hilberg et al. 2001), tendon stiffness (Cruz-Montecinos et al. 2019b), and impaired proprioception (Hilberg et al. 2001). In addition, static postural control and lower limb kinematics during gait are affected in PWHA (Cruz-Montecinos et al. 2017; Gallach et al. 2008; García-Massó et al. 2019; Lobet et al. 2010, 2012; Stephensen et al. 2012). However, we know little about the consequences of these changes on neuromuscular control of gait. Applying a muscle synergy approach (Steele et al. 2015; Tresch et al. 2006), it was found that, during gait in PWHA compared to healthy controls, the total variance of electromyography (EMG) accounted for by one muscle synergy was higher (Cruz-Montecinos et al. 2019a). This result suggests increased co-contraction of antagonistic muscles. However, the muscle synergy analysis does not yield information about potential differences in the neural control of individual muscles and changes in co-contraction between antagonistic muscle pairs (Groote et al. 2014).

The coordination between antagonistic muscles during dynamic activities such as gait is key in the understanding of the progression of joint degeneration. In knee osteoarthritis (OA), the co- contraction between knee flexors extensors during the stance phase has been related to the progression of knee OA and greater cartilage loss (Hodges et al. 2016; Hubley-Kozey et al. 2009). A higher co- contraction between superficial ankle plantar flexors and dorsiflexors during the stance phase has been also reported in people with ankle OA (Doets et al. 2007; von Tscharner and Valderrabano 2010). Similar changes in co-contraction between muscles crossing knee and ankle joints during gait may be expected in PWHA. A better understanding of the individual muscle activity patterns and co-

contraction between antagonists during gait in PWHA may be used to improve rehabilitation strategies aimed at increasing muscle strength (Calatayud et al. 2020), for neuromuscular re-education (Preece et al. 2016), or to improve the feedback strategies during gait (Booth et al. 2019), as well the orthopedic surgeries (Rodriguez-Merchan 2012). The aim of the present study was to assess if the neural control of individual muscles, coordination between antagonistic muscle pairs, and joint kinematics during gait are affected in PWHA. For this purpose, EMG activity of several leg muscles and joint kinematics were recorded in PWHA and a control group (CG). Waveforms were compared using the time-series analysis through statistical parametric mapping (SPM) (Pataky 2010).

MATERIALS AND METHODS

Participants

This study was approved by the local ethical committee and conducted in agreement with the Declaration of Helsinki. The data of the present study have been used for a previous paper, which addressed a different research question, using other data analysis methods (i.e., muscle synergies) and focused on different outcome measures (Cruz-Montecinos et al. 2019a). All participants were informed about the purpose and procedures of the project and gave their written informed consent to participate in the study. Fourteen PWHA and 13 healthy control subjects were recruited (for the characteristics of each group, see Table 1).

People With Haemophilic Arthropathy

Inclusion criteria: Males, diagnostic haemophilia A or B severe and moderate, haemophilic arthropathy with a minimum of 1 point (in knee or ankle) of the

radiological Pettersson score assessed with X-ray examination, over 18 years of age and under 45 years, passive range of motion (ROM) of the knee >60◦ and >20◦ in ankle [both values correspond to approximately 30 and 40% of the normal ROM (Soucie et al. 2011), respectively], prophylaxis treatment with deficient factor (i.e., VIII or IX), and body mass index less than 30. Exclusion criteria: History of hip, knee or ankle arthroplasty, equinus foot, inability to walk without an assistive device (e.g., walker, cane), history of muscle or joint bleeding in lower limbs in the last 2 months, chronic cardiac and/or respiratory pathology and neurological disease.

Control Subjects

Inclusion criteria were the following: male, over 18 years of age and under 45 years, no haemophilia, and body mass index lower than 30. Exclusion criteria were the following: a Haemophilia Joint Health Score (see below) > 0, traumatic injuries; signs or symptoms of injury or symptomatic arthritis to the trunk, lower back, and lower limb within the past 3 months; which a findings of the Alt-man's criteria for knee OA (i.e., morning stiffness < 30 min, crepitus, bony tenderness, bony enlargement, palpable warmth) (Altman et al. 1986; Na and Buchanan 2019); history of musculoskeletal surgery in the lower limb and spine; scoliosis; history of acute or chronic musculoskeletal disorders; cardiac and/or respiratory pathology; and neurological disease.

TABLE 1 | Clinical characteristics between groups.

Characteristic between groups	CG = 13	PWHA = 14	*p* value
Age (years)	28.4 ± 6.1	28.4 ± 6.6	0.991
Body mass (kg)	75.5 ± 8.0	73.9 ± 11.6	0.687
Height (cm)	175.6 ± 4	171.7 ± 8	0.115
Body mass index	24.4 ± 1.9	25.0 ± 3.4	0.593
Pain during 30 m walk	0 [0 0]	1 [0 5]	**0.002**
Duration of pain (years)	NA	7.5 [5 20]	NA
Pain medication	NA	4/14	NA
Opioids medication	NA	0/14	NA
Physical activity (>150 min/week)	7/13	4/14	0.345

People with haemophilic arthropathy (PWHA; n = 14). Control group (CG; n = 13). Parametric distribution: mean ± SD. Non-parametric distribution: median [range]. NA, not applicable. Significant differences ($p < 0.05$) are in bold.

Surface Electromyography Protocol for the Lower Limb

In PWHA, the limb with the highest points on the radiological Pettersson score was selected. In CG, the dominant limb was assessed. Leg dominance was assessed by asking the subjects which leg they would use to kick a ball (Chia Bejarano et al. 2017). After shaving and cleaning the skin with alcohol, surface electrodes (Ag–AgCl, Kendall H124SG) were placed (interelectrode spacing 2 cm) on the following muscles according to SENIAM guidelines (Hermens et al. 2000): medial gastrocnemius (MG), lateral gastrocnemius (LG), soleus (SOL), tibialis anterior (TA), vastus lateralis (VL), medialis (VM), rectus femoris (RF), semitendinosus (ST), biceps femoris (BF), gluteus maximus (GMAX), and gluteus medius (GMED). Activity patterns of these muscles were measured using a wireless EMG system (MyoSystem DTS, Noraxon USA Inc., Scottsdale, CA, United States), with a sampling rate of 1,500 Hz. Heel strike was detected by a synchronized wireless pressure sensor (Noraxon USA Inc., Scottsdale, CA, United States) placed underneath the heel of the foot (Figure 1).

Kinematics of the Lower Limb

Based on inertial measurement units (IMUs), the sagittal kinematics of the hip, knee, and ankle joints were assessed. Four IMU sensors (Xsens, Enschede, Netherlands) were positioned between posterior superior iliac spines, the lateral face of the thigh within the proximal third, lateral face of the shank within the distal third close to the lateral malleolus, and the midfoot (Cutti et al. 2010). The sensors placed on sacrum, thigh, and shank were fixed with the fixation system provided by the company (Xsens, Enschede, Netherlands). The sensor placed on midfoot was fixed by adhesive elastic taping (Leukotape K, BSN Medical, Hamburg, Germany) with sufficient tension to avoid movement artifacts (Figure 1). The Xsens system and the traditional camera-based optical motion capture systems have reported similar flexion–extension hip, knee, and ankle waveforms (coefficient of multiple correlation > 0.96) for hip, knee, and ankle angle during overground walking, with average angle estimation errors of 2.15, 1.87, and 2.47◦, respectively (Zhang et al. 2013). Data were collected at a sampling frequency of 75 Hz. The IMUs and EMG/pressure sensor were synchronized through a trigger pulse.

Experimental Protocol

Each subject was invited to walk barefoot overground at their preferred velocity and the CG also walked at a slower velocity (1.0 m/s) similar to that of the mean preferred velocity in PWHA. Two velocities were tested for the CG because joint kinematics and muscle activity patterns are dependent on gait velocity (Kirtley et al. 1985; Tirosh and Sparrow 2005). For the CG, the slow velocity was practiced three times for 10 m. Subsequently, each subject walked for 30 m twice (Figure 1). Mean velocity was assessed by dividing total distance by total time. To reduce the

risk of muscular and intramuscular bleeding during the experimental procedures, 2 min of rest in between tests were allowed. The PWHA received prophylactic treatment 1 and 2 h before the experiment.

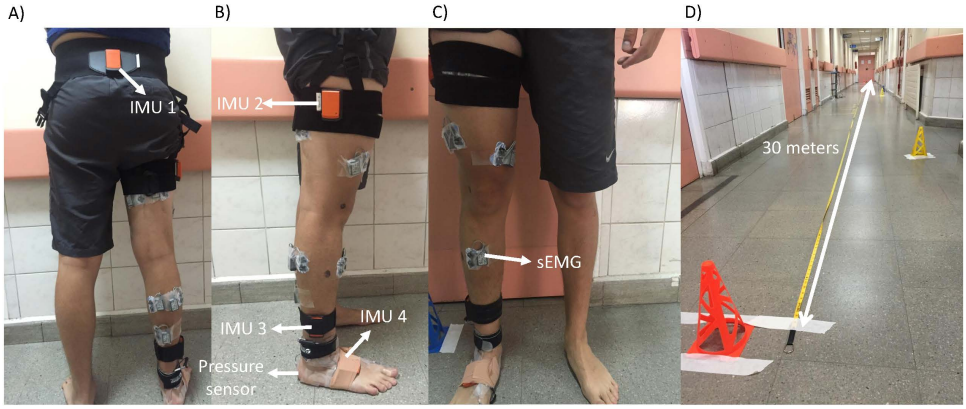


FIGURE 1 | Sensors locations and 30 m used corridor. **(A)** Back view. **(B)** Lateral view. **(C)** Frontal view. **(D)** Walking assessment corridor. Surface electromyography (sEMG), inertial sensor positioned in pelvis (IMU 1), inertial sensor positioned in lateral face of thigh (IMU 2), inertial sensor positioned lateral face of the shank (IMU 3), and inertial sensor positioned in midfoot (IMU 4).

Data Analysis

For EMG and kinematic analysis, we used Matlab software 2016 (Statistics and Machine Learning Toolbox, MathWorks, Inc., Natick, MA, United States).

EMG Signal Processing

In each subject, a total of 20 cycles were used for the final analysis. First, a bandpass filter (20–500 Hz, Butterworth, fifth order) was applied. The EMG signals during each step cycle were time normalized to 0– 100%. To assess EMG amplitudes, the EMG signals were rectified using Hilbert transformation and smoothed with a low-

pass filter at 6 Hz (Hubley-Kozey et al. 2006; Rutherford et al. 2017, 2011). The amplitude for each muscle was normalized to the maximum value of all included steps (i.e., 20 cycles) separately for each group and velocity (i.e., CG during preferred velocity, CG during slow velocity, and PWHA during preferred velocity). This procedure indicates at what periods during the gait cycle that the muscle is relatively more active (Benoit et al. 2003; Cronin et al. 2015). With this method, differences between injured and non-injured legs in relative intensity and timing of EMG activity have been reported for several clinical populations (i.e., ankle OA, ankle arthrodesis, and Achilles tendon surgery) (Doets et al. 2007; Suydam et al. 2015; Wu et al. 2000). The normalized EMG signals were used to calculate the co-contraction index (CCI) at each point of the gait cycle, providing a time-series curve to describe the temporal and magnitude components of the EMG signals based on the following formula (Knarr et al. 2012; Rudolph et al. 2000),

$$CCI_i = \frac{LEMG_i}{HEMG_i}(LEMG_i + HEMG_i),$$

where i is the point of the gait cycle, LEMG is the normalized magnitude of the EMG for the less active muscle, and HEMG is the normalized magnitude of the EMG for the most active muscle (Knarr et al. 2012). The CCI was calculated for the following antagonistic muscle pairs crossing the ankle (MG-TA, LG-TA, and SOL-TA) and knee joint (VL-BF, VM-ST, LG-VL, and MG-VM). CCI goes from 0 to 2, where a CCI value of 2 indicates the maximum normalized value for both LEMG and HEMG.

The EMG bursts were identified using the k-means cluster analysis applied on the bandpass filter and rectified signal using Hilbert transformation. Three clusters were assigned to k-means cluster analysis, where the lowest cluster reflects inactivity (Bernabei et al. 2017; Den Otter et al. 2006). Then, EMG burst on-and

offset were identified, using the following criteria: every burst shorter than 5 ms was discarded; bursts separated by <125 ms were considered the same burst (De la Fuente et al. 2018a; Merlo et al. 2003). For the final analysis, the mean of the 20 cycles was used to represent the muscle temporal and intensity patterns, co-contraction, and the on-/offset of individual muscles for each subject. The Euler angles of each IMU were determined using the quat2angle function in Matlab Software (version 2016; MathWorks, Inc., Natick, MA, United States) using as input the IMU quaternion orientation.

Flexion–Extension Angle Estimation and Temporal–Spatial Parameter During Gait

To define the sensor to segment alignment, subjects were asked to stand still in a neutral position with their feet parallel, one-foot width apart, and legs and back straight for 10 s (Laudanski et al. 2013). Each axis was reset to define (x, y, z) coordinate. Based on recommendations of the International Society of Biomechanics, the x-axis was defined as the anteroposterior axis, y-axis as the vertical axis, and z-axis as the mediolateral axis (Wu et al. 2002). To identify the flexion–extension plane, a sequence of bipodal independent flexions of trunk, hip, knee, and ankle were used as reference before starting the protocol. The hip, knee, and ankle angles were defined as flexion– extension angles of the distal body segment with respect to the proximal body segments (Laudanski et al. 2013). Low-pass Butterworth filters were applied to the IMU joint angle data, with a cut-off frequency of 10 Hz. The segmentation of kinematics signals were determined through gait events signifying heel strike and toe-off from the angular velocity in z-axis obtained from the IMU sensor of the shank (De Vroey et al. 2018a). This method has a strong agreement and excellent correlations with temporal parameters of gait evaluated by means of a camera-based motion capture system

(De Vroey et al. 2018a). The kinematic signals during each step were normalized to total stride time determined with IMU sensor of the shank. For the final analysis, the mean of the same 20 cycles selected for the EMG analysis were used to represent the kinematics and temporal parameters of gait. To assess the total time of the 30-m walking test, the acceleration of the IMU sensor of the shank was used.

Clinical Assessments for PWHA

To assess the intensity of pain (scale 0–10 points) during barefoot walking, the Visual Analogue Scale was used. A physical therapist with 10 years of experience in haemophilia rehabilitation (CCM) applied the Haemophilia Joint Health Score 2.1 (HJHS) (Gouw et al. 2019; Sun et al. 2014). The HJHS 2.1 score is used to assess the health status of the joints most commonly affected by bleeding in haemophilia: the knees, ankles, and elbows. This scale consists of eight items per joint (scale 0–20), evaluating (1) joint swelling (0–3 points), (2) duration of swelling (0–1 pts), (3) muscle atrophy (0–2), (4) strength (0–4), (5) crepitus on motion (0–2 points),(6) flexion loss (0–3 points), (7) extension loss (0– 3 points), and (8) pain (0–2 points) (Sun et al., 2014). The radiological Pettersson score (scale 0–13) (Pipe and Valentino 2007) was assessed by a medical doctor (FQ) with more than 30 years of experience in haemophilia.

Statistical Analysis

A priori power analysis conducted in G Power (3.1.9.2 version) software (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) showed that 13 subjects per group this design were sufficient to obtain a statistical power of 0.80 at a large effect size (Cohen's d = 1.03; Cruz-Montecinos et al. 2019a), with an alpha = 0.05. For all statistical analysis, we used Matlab 2016 (MathWorks, Inc., Natick, MA,

United States). The normality of data was evaluated through the Shapiro–Wilk test. For all comparison, the alpha level was set at 0.05. To compare the clinical characteristics between groups, the independent samples *t* test was used for age, body mass, height, and body mass index. The Wilcoxon rank-sum test and the chi-squared test was used for pain during walking and physical activity (> 150 min/week). Data are expressed as the mean ± standard deviation for normal distribution and median (range) for no-normal distribution.

RESULTS

Clinical Characteristics

All PWHA were diagnosed with haemophilia A (11 severe and 3 moderate) (for characteristics between groups, see Table 1). The joint damage in PWHA showed similar values of the clinical and radiological status for the knee and ankle joint (see Table 2).

Table 2. Clinical joint assessment.

Clinical joint assessment in PWHA (n=14)	Joint	Points
Haemophilia Joint Health Score (0-20 pts)	Ankle	7.1 ± 3.4
	Knee	6.1 ± 5.2
Radiological Pettersson score (0-13 pts)	Ankle	5.9 ± 4.1
	Knee	3.4 ± 3.4

Joint assessment for people with haemophilic arthropathy (PWHA). Data are expressed as mean and standard deviation.

Muscle Activity Patterns and Co-contraction

Comparing the muscle activity patterns between groups at their preferred velocity revealed different activity patterns in the ankle plantar flexors and knee flexors. In the muscles crossing the ankle, PWHA showed a relatively higher activation of MG during the swing phase (at ~70% of the gait cycle; p = 0.008, d = 1.26), a relatively higher activation of LG during the stance phase (at around 10–20% of the gait cycle; p = 0.001, d = 1.56) (Figure 2), and a relatively lower activation of SOL during the propulsion phase (at ~ 40% of the gait cycle; p = 0.038, d = 1.35).

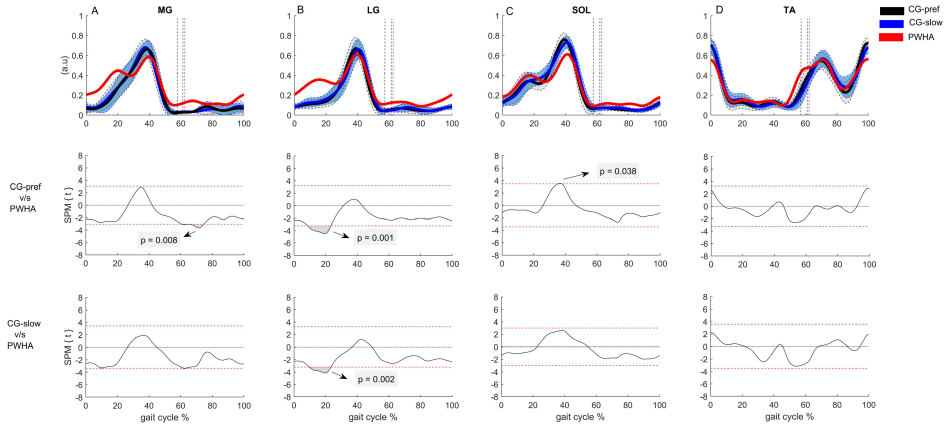


FIGURE 2 | Comparison between groups of surface electromyography of muscles crossing the ankle. **(A)** Medial gastrocnemius (MG). **(B)** Lateral gastrocnemius (LG). **(C)** Soleus (SOL). **(D)** Tibialis anterior (TA). **(Top row**) Surface electromyography of muscles crossing the ankle of people with haemophilic arthropathy (PWHA; *n* = 14) at their preferred velocity (red), the control group (*n* = 13) at their preferred velocity (CG-pref, black), and during the slow velocity condition (CG-slow, blue). Data are plotted as a function of normalized step time (0, heel strike) and expressed as mean with 95% confidence interval for CG. For the 95% confidence interval of PWHA, see figures 1 and 2 in the appendix. Vertical dashed lines indicate transition from stance to swing phase (black, CG-pref; blue, CG-slow; red, PWHA). **(Bottom two rows)** Time-dependent *t* values of the statistical parametric mapping (SPM) for groups comparison. Horizontal red dashed line indicates *p* = 0.05 level. Gray zones indicate regions with statistically significant differences. a.u, Arbitrary unit.

In the muscles crossing the knee, PWHA showed a relatively higher activation of ST during the stance phase (at ~10% of the step cycle; $p = 0.001$, $d = 1.39$) and lower at the end of the swing phase (at ~ 95% of the gait cycle; $p = 0.040$, $d = 1.31$) (Figure 3). For the muscles crossing the hip, no differences between group were found for GMAX and GMED (Figure 4).

For the co-contraction of pair muscles crossing the ankle, PWHA showed higher CCI during the stance phase for MG-TA (at ~ 20% of the step cycle; $p = 0.010$, $d = 1.34$) and LG-TA (at ~ 20% of the step cycle; $p < 0.001$, $d = 1.46$) and during the swing phase for MG-TA (at ~ 70% of the step cycle; $p = 0.032$, $d = 1.32$) and LG-TA (at ~ 60% of the step cycle; $p = 0.016$, $d = 1.06$) (Figure 5). No difference between groups was found for SOL-TA (Figure 5). For the co-contraction of pair muscles crossing the knee, PWHA showed a higher CCI during the stance phase for VL-BF (at ~ 10% of the step cycle; $p = 0.008$, $d = 1.46$), VM-ST (at ~ 20% of the step cycle; $p < 0.001$, $d = 1.58$), VL-LG (at ~ 20% of the step cycle; $p < 0.001$, $d = 1.34$), VM-MG (at ~ 20% of the step cycle; $p = 0.003$, $d = 1.40$), and during the swing phase for VM-ST (at ~ 70% of the step cycle; $p = 0.011$, $d = 1.53$) and VM- MG (at ~ 70% of the step cycle; $p = 0.015$, $d = 1.36$) (Figure 6).

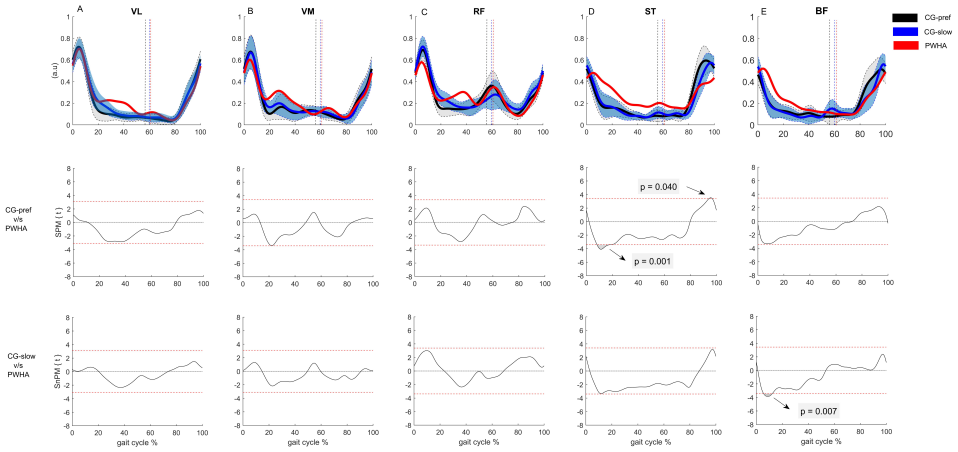


FIGURE 3 | Comparison between groups of surface electromyography of muscles crossing the knee. **(A)** Vastus lateralis (VL). **(B)** Vastus medialis (VM). **(C)** Rectus femoris (RF). **(D)** Semitendinosus (ST). **(E)** Biceps femoris (BF). **(Top row)** Surface electromyography of muscles crossing the knee of people with haemophilic arthropathy (PWHA; *n* = 14) at their preferred velocity (red), the control group (*n* = 13) at their preferred velocity (CG-pref, black), and during the slow velocity condition (CG-slow, blue). Data are plotted as a function of normalized step time (0, heel strike) and expressed as mean with 95% confidence interval for CG. For the 95% confidence interval of PWHA, see figures 1 and 2 in the appendix. Vertical dashed lines indicate transition from stance to swing phase (black, CG-pref; blue, CG-slow; red, PWHA). **(Bottom two rows)** Time-dependent *t* values of the statistical parametric mapping (SPM) for groups comparison. Horizontal red dashed line indicates *p* = 0.05 level. Gray zones indicate regions with statistically significant differences. a.u, Arbitrary unit.

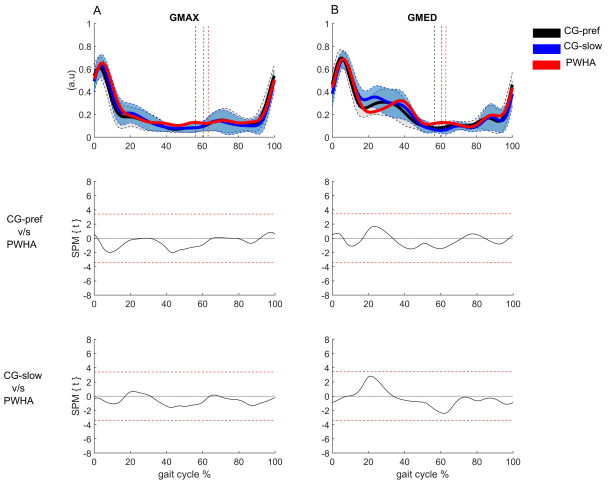


FIGURE 4 | Comparison between groups of surface electromyography of muscles crossing the hip. **(A)** Gluteus maximus (GMAX). **(B)** Gluteus medius (GMED). **(Top row)** Surface electromyography of muscles crossing the hip of people with haemophilic arthropathy (PWHA; *n* = 14) at their preferred velocity (red), the control group (*n* = 13) at their preferred velocity (CG-pref, black), and during the slow velocity condition (CG-slow, blue). Data are plotted as a function of normalized step time (0, heel strike) and expressed as mean with 95% confidence interval for CG. For the 95% confidence interval of PWHA, see figures 1 and 2 in the appendix. Vertical dashed lines indicate transition from stance to swing phase (black, CG-pref; blue, CG- slow; red, PWHA). **(Bottom two rows)** Time-dependent *t* values of the statistical parametric mapping (SPM) for groups comparison. Horizontal red dashed line indicates *p* = 0.05 level. Gray zones indicate regions with statistically significant differences. a.u, Arbitrary unit.

Comparing muscle activity patterns between groups at the same velocity revealed

different activity patterns in the plantar flexors and knee flexors. In the muscles crossing the ankle, PWHA showed relatively higher activation of LG during the stance phase (at ~ 10–20% of the gait cycle; p = 0.002, d = 1.46) (Figure 2). In the muscles crossing the knee, PWHA showed relatively higher activation of BF during the stance phase (at~ 10% of the step cycle; p = 0.007, d = 1.44) (Figure 3). For the

muscles crossing the hip, no differences between group were found for GMAX and GMED (Figure 4).

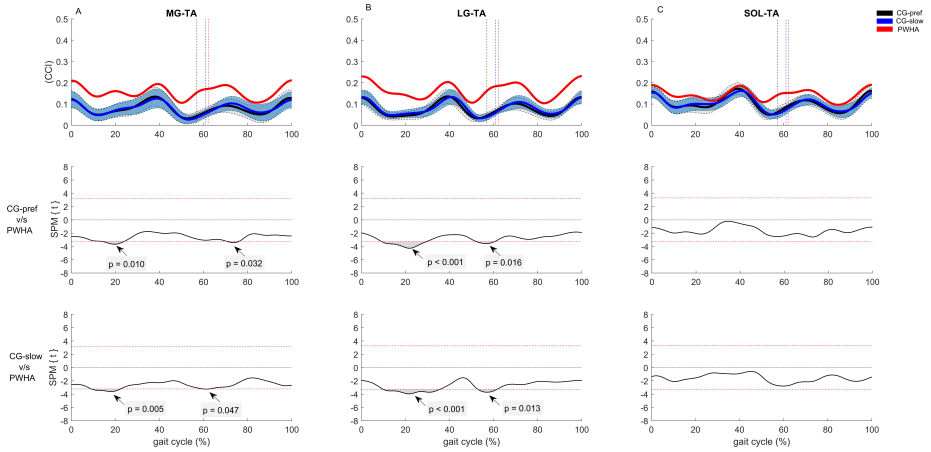


FIGURE 5 | Co-contraction of muscles crossing the ankle during gait. **(A)** Con-contraction index (CCI) between medial gastrocnemius and tibialis anterior (MG-TA). **(B)** CCI between lateral gastrocnemius and tibialis anterior (LG-TA). **(C)** CCI between soleus and tibialis anterior (SOL-TA). **(Top row)** CCI of muscles crossing the ankle of people with haemophilic arthropathy (PWHA; *n* = 14) at their preferred velocity (red), the control group (*n* = 13) at their preferred velocity (CG-pref, black), and during the slow velocity condition (CG-slow, blue). Data are plotted as a function of normalized step time (0, heel strike) and expressed as mean with 95%confidence interval for CG. Vertical dashed lines indicate transition from stance to swing phase (black, CG-pref; blue, CG-slow; red, PWHA). **(Bottom two rows)** Time-dependent *t* values of the statistical parametric mapping (SPM) for groups comparison. Horizontal red dashed line indicates *p* = 0.05 level. Gray zones indicate regions with statistically significant differences. a.u, Arbitrary unit.

For the co-contraction of pair muscles crossing the ankle, PWHA showed a higher CCI during the stance phase for MG-TA (at ~ 20% of the step cycle; p = 0.005, d = 1.33) and LG-TA (at ~ 20% of the step cycle; p < 0.001, d = 1.41) and during the swing phase for MG-TA (at ~ 60% of the step cycle; p = 0.047, d = 0.918) and LG-TA (at ~ 60% of the step cycle; p = 0.013, d = 1.24) (Figure 5). No difference between group was found for the pair SOL- TA (Figure 5). For the co-contraction of pair muscles crossing the knee, PWHA showed a higher CCI during the stance phase for VL-BF (at ~ 10% of the step cycle; p = 0.015, d = 1.47), VM-ST (at ~ 20% of the step

cycle; p = 0.006, d = 1.41), VL-LG (at ~ 10% of the step cycle; p = 0.018, d = 1.30; at ~ 40% of the step cycle; p = 0.042, d = 1.29), and VM-MG (at ~ 20% of the step cycle; p = 0.013, d = 1.30) (Figure 6).

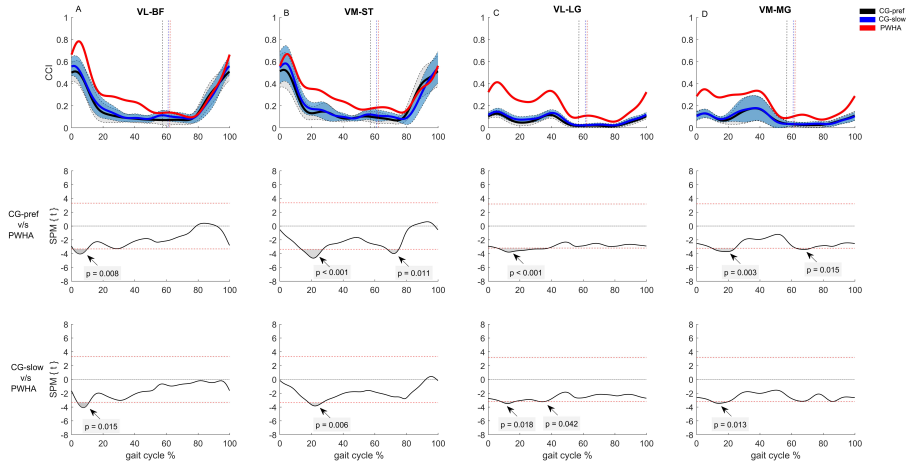


FIGURE 6 | Co-contraction of muscles crossing the knee during gait. **(A)** Con-contraction index (CCI) between vastus lateralis and biceps femoris (VL-BF). **(B)** CCI between vastus medialis and semitendinosus (VM-ST). **(C)** CCI between vastus lateralis and lateral gastrocnemius (VL-LG). **(D)** CCI between vastus medialis and medial gastrocnemius (VM-ST). **(Top row)** CCI of muscles crossing the knee of people with haemophilic arthropathy (PWHA; *n* = 14) at their preferred velocity (red), the control group (*n* = 13) at their preferred velocity (CG-pref, black), and during the slow velocity condition (CG-slow, blue). Data are plotted as a function of normalized step time (0, heel strike) and expressed as mean with 95% confidence interval for CG. Vertical dashed lines indicate transition from stance to swing phase (black, CG-pref; blue, CG-slow; red, PWHA). **(Bottom two rows)** Time-dependent *t* values of the statistical parametric mapping (SPM) for groups comparison. Horizontal red dashed line indicates *p* = 0.05 level. Gray zones indicate regions with statistically significant differences. a.u, Arbitrary unit.

Timing and Duration of EMG Activity Between Groups

Comparing the onset/offset activation pattern of individual muscles between groups at their preferred velocity revealed different timing and duration in EMG activity in various muscles. In the muscles crossing the ankle, the MG and LG muscles in PWHA showed an earlier onset during the stance phase and longer total duration of activity (Table 3 and Figure 7). In the muscles crossing the hip, the GMAX muscle showed a later offset during the stance phase and a longer total duration of activity (Table 3 and Figure 7).

Differences in timing and duration of EMG activity were also found when comparing the onset/offset activation pattern of individual muscles between groups at the same velocity. In the muscles crossing the ankle, PWHA showed a longer total duration of activity of MG and LG (Table 3). In the muscles crossing the knee, the VM, ST, and BF showed a later offset during the stance phase in PWHA and the longer total duration of activity of VM and ST (Table 3 and Figure 7).

TABLE 3 | Statistical results (*p* values) and effect size (ES) for EMG burst onset–offset and burst duration.

	Muscle	Variable	PWHA (n=14) vs. CG-pref (n=13)	PWHA (n=14) vs. CG-slow (n=13)
			p value (*d*)	*p* value (*d*)
Muscles crossing the ankle	Medial gastrocnemius	Onset	**0.034 (0.91)**	0.103 (0.69)
		Offset	0.198 (0.73)	0.610 (0.59)
		Duration	**0.001 (1.53)**	**0.005 (1.21)**
	Lateral gastrocnemius	Onset	**0.006 (1.30)**	0.058 (0.72)
		Offset	0.061(0.76)	0.107 (0.64)
		Duration	**<0.001 (1.54)**	**0.007 (1.09)**
	Soleus	Onset	0.636 (0.05)	0.269 (0.32)
		Offset	0.942 (0.08)	0.680 (0.11)
		Duration	0.645 (0.13)	0.396 (0.22)
	Tibialis anterior	Onset	0.198 (0.32)	0.089 (0.49)
Muscles crossing the knee		Offset	0.899 (0.05)	0.649 (0.18)
		Duration	0.396 (0.23)	0.437 (0.22)
	Vastus lateralis	Onset	0.752 (0.38)	0.627 (0.37)
		Offset	**0.011 (1.21)**	0.167 (0.60)
		Duration	**0.027 (1.05)**	0.369 (0.41)
	Vastus medialis	Onset	0.752 (0.35)	0.512 (0.36)
		Offset	**0.002 (1.53)**	**0.014 (1.11)**
		Duration	**0.005 (1.30)**	**0.020 (0.96)**
	Rectus femoris	Onset	0.216 (0.64)	0.145 (0.80)
		Offset	0.275 (0.67)	0.577 (0.12)
		Onset (second burst)	0.209 (0.60)	**0.054 (1.19)**
		Offset (second burst)	0.998 (0.01)	0.840 (0.14)
		Duration	0.716 (0.06)	0.790 (0.08)
	Semitendinosus	Onset	0.396 (0.53)	0.528 (0.49)
		Offset	**0.031 (0.97)**	**0.003 (1.25)**
		Duration	0.069 (0.61)	**0.035 (0.75)**
	Biceps femoris	Onset	0.152 (0.75)	0.065 (0.72)
Muscles crossing the hip		Offset	**0.001 (1.64)**	**0.002 (1.42)**
		Duration	**0.023 (1.08)**	0.077 (0.81)
	Gluteus maximus	Onset	0.647 (0.18)	0.409 (0.32)
		Offset	**0.003 (1.17)**	0.055 (0.80)
		Duration	**0.003 (1.22)**	0.073 (0.91)
	Gluteus medius	Onset	0.790 (0.08)	0.903 (0.16)
		Offset	0.369 (0.58)	0.577 (0.40)
		Duration	0.409 (0.40)	0.610(0.20)

Comparison between the control group during preferred (CG−pref; n = 13) and slow walking velocity (CG−slow; n = 13) and people with haemophilic arthropathy (PWHA; n = 14) during preferred walking velocity. The second burst of rectus femoris muscle during stance to swing transition was present in only part of the subjects: 11/13 for the CG-pref, 6/13 for CG-slow, and 9/14 in PWHA. Effect size (d) Significant differences ($p < 0.05$) are in bold.

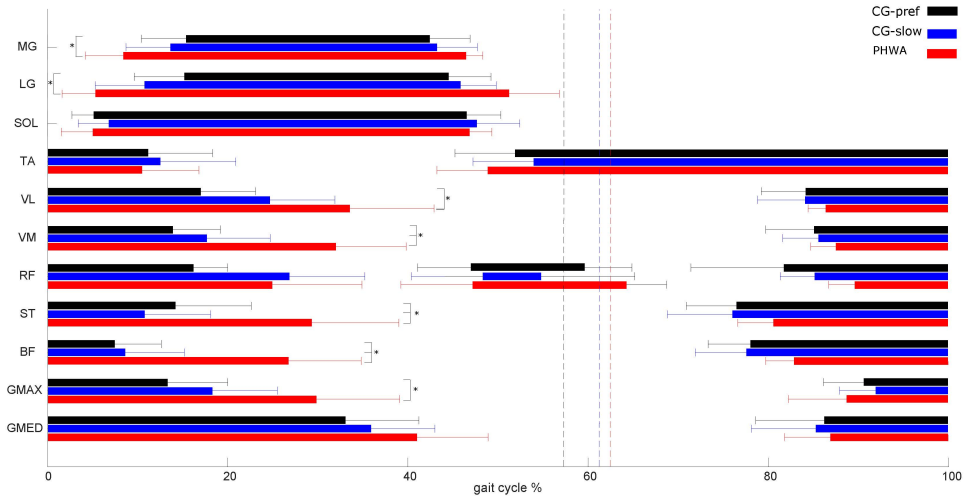


FIGURE 7 | Electromyography (EMG) burst on- and offset during gait. EMG burst on- and offset during gait of people with haemophilic arthropathy (PWHA; *n* = 14) at their preferred velocity (red), the control group (*n* = 13) at their preferred velocity (CG-pref, black), and during the slow velocity condition (CG-slow, blue). The second burst of rectus femoris muscle during stance to swing transition was present in only part of the subjects: 11/13 for the CG-pref, 6/13 for CG-slow, and 9/14 in PWHA. Data are plotted as a function of normalized step time (0, heel strike) and expressed as mean with 95% confidence interval for CG. Vertical dashed lines indicate transition from stance to swing phase (black, CG-pref; blue, CG-slow; red, PWHA). Medial gastrocnemius (MG), lateral gastrocnemius (LG), soleus (SOL), tibialis anterior (TA), vastus lateralis (VL), vastus medialis (VM), rectus femoris (RF), semitendinosus (ST), biceps femoris (BF), gluteus maximus (GMAX), and gluteus medius (GMED). Data are expressed as mean and 95% confidence interval.

Kinematic and the Temporal Gait Parameters

Comparing the kinematics between groups at their preferred velocity, the SPM analysis showed significant differences of the hip, knee, and ankle joints (Figure 8). In PWHA, the hip joint showed the lower amplitude of flexion during the swing phase (at around 60–90% of the cycle; p = 0.009, d = 1.59) and lower ROM (mean

differences of 5.6◦, p = 0.008, d = 1.12) (Table 4). The knee joint in PWHA showed a lower amplitude of flexion during the transition from stance to swing phase (at around 53–80% of the cycle; p = 0.003, d = 1.47) and lower ROM (mean differences of 14.4◦, p < 0.001, d = 1.85) (Table 4). The ankle joint in PWHA showed a lower amplitude of plantar flexion during stance (at around 65–68% of the gait cycle; p = 0.047, d = 1.22) and lower ROM (mean difference of 5.6◦, p = 0.008, d = 1.12) (Table 4).

TABLE 4 | Temporal variables and total range of motion (ROM) during walking.

Variables	CG-pref	CG-slow	PWHA	PWHA (n=14) vs. CG-pref (n=13)	PWHA (n=14) vs. CG-slow (n=13)
				p-value	p-value
Velocity in 30 m (m/s)	1.2 ± 0.2	1.0 ± 0.2	1.1 ± 0.1	**0.047**	0.146
Time cycle (s)	1.1 ± 0.1	1.2 ±0.1	1.1 ± 0.1	0.480	**0.010**
CV of time cycle %	1.7 ± 0.5	2.0 ± 0.6	2.6 ± 0.7	**0.001**	**0.014**
Stance time %	57.3 ± 3.0	61.3 ± 3.6	62.4 ± 3.8	**0.001**	0.498
ROM of hip (degrees)	35.4 ± 4.9	33.3 ± 5.5	29.8 ± 5.1	**0.008**	0.096
ROM of knee (degrees)	53.7 ± 7.1	51.2 ± 5.1	39.3 ± 8.3	**<0.001**	**<0.001**
ROM of ankle (degrees)	27.3 ± 5.8	25.7 ± 4.4	20.9 ± 7.0	**0.015**	**0.043**

Comparisons between the control group during preferred (CG-pref) and slow walking velocity (CG-slow) and people with haemophilic arthropathy (PWHA) during preferred walking velocity. Coefficient variation (CV). Effect size (d). Data are expressed as mean and standard deviation. Significant differences (p < 0.05) are in bold.

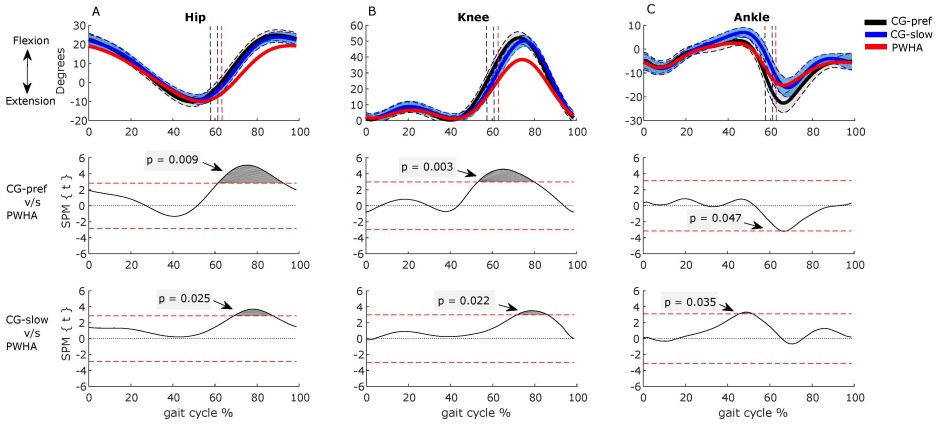


FIGURE 8 | Comparison between groups of kinematics during gait. **(A)** Hip. **(B)** Knee. **(C)** Ankle. **(Top row)** Kinematics of people with haemophilic arthropathy (PWHA; *n* = 14) at their preferred velocity (red), the control group (*n* = 13) at their preferred velocity (CG- pref, black), and during the slow velocity condition (CG-slow, blue). Data are plotted as a function of normalized step time (0, heel strike) and expressed as mean with 95% confidence interval for CG. Vertical dashed lines indicate transition from stance to swing phase (black, CG-pref; blue, CG-slow; red, PWHA). **(Bottom two rows)** Time-dependent *t* values of the statistical parametric mapping (SPM) for groups comparison. Horizontal red dashed line indicates *p* = 0.05 level. Gray zones indicate regions with statistically significant differences. Data are expressed as mean and 95% confidence interval.

The knee joint in PWHA showed a lower amplitude of flexion during the transition from stance to swing phase (at around 53–80% of the cycle; p = 0.003, d = 1.47) and lower ROM (mean difference of 14.4◦, p < 0.001, d = 1.85) (Table 4). The ankle joint in PWHA showed a lower amplitude of plantar flexion during stance (at around 65–68% of the gait cycle; p = 0.047, d = 1.22) and lower ROM (mean difference of 7.5◦, p = 0.015, d = 1.31) (Table 4).

Regarding temporal gait parameters, the preferred velocity of PWHA was lower than that of the CG (difference, 0.1 m/s; p = 0.044, d = 0.80). PWHA compared to CG at preferred velocity showed a similar cycle time (difference, 0 s; p = 0.480, d = 0.28), a higher coefficient of variation of time cycle (difference, 0.9%; p = 0.001, d= 1.47) and longer stance time (difference, 5.1%; p = 0.002, d = 1.44) (Table 4).

Comparing the kinematics between groups at the same velocity, the SPM analysis showed a significant difference in hip, knee, and ankle angles (Figure 8). In PWHA, the hip joint showed the lower amplitude of ROM during the swing phase (at around 69–88% of the cycle; p = 0.025, d = 1.27); however, no difference between PWHA and controls was found in ROM of hip (Table 4). The knee joint in PWHA showed a lower amplitude of ROM during the swing phase (at around 72–87% of the cycle; p = 0.022, d = 1.27) and a lower ROM (difference of 11.9◦, p = 0.002, d = 1.71) (Table 4). The ankle joint in PWHA showed a lower amplitude of dorsi-flexion during the stance phase (at around 45–52% of gait cycle p = 0.035, d = 1.24) and a lower ROM (difference of 5.9◦, p = 0.006, d = 1.16) (Table 4). The preferred velocity of PWHA was similar than the slow velocity of CG (difference, 0.1 m/s; p = 0.203, d = 0.58). PWHA compared to CG at the same velocity showed a lower cycle time (difference, 0.1 s; p = 0.010, d = 1.07), a higher coefficient of variation of cycle time (difference, 0.6%; p = 0.010, d = 1.02) and a similar stance time (difference, 1.1%; p = 0.544, d = 0.26) (Table 4). These results indicate that the kinematics of the leg joints in PWHA differs from that of the CG.

DISCUSSION

The aim of the present study was to assess if the neural control of individual muscles, coordination between antagonistic muscle pairs, and joint kinematics during gait are affected in PWHA. The main results of our study are that PWHA differs from controls with regard to the following: (i) EMG amplitudes of the triceps surae and hamstrings muscles, (ii) level of co-contraction for several antagonistic muscles crossing the ankle and knee (i.e., MG-TA, LG-TA, VL-BF, VM-ST, LG-VL, and MG-VM), (iii) timing and duration of EMG activity of several muscles (i.e., MG, LG, VL, VM, ST, BF, and GMAX), and (iv) range of motion of in the ankle, knee, and hip joints. To the authors' current knowledge, this study is the first to report that the muscle activity and antagonistic co- contraction patterns and the temporal on–off activation pattern of several leg muscles during gait in PWHA differ from those in CG.

Differences in Neuromuscular Control

In the muscles crossing the ankle, the LG of PWHA showed relatively higher activation during the first half of the stance phase and an earlier onset for MG and LG. The different activation pattern of LG (and to a lesser extent also MG) observed in PWHA is similar to that reported for people with an elongated Achilles tendon following surgical reconstruction (Suydam et al. 2015)and that for people with ankle OA (Doets et al. 2007). The earlier activation of MG and LG muscles and increased amplitude during the first half of the stance phase make the activation pattern similar to that of SOL muscle (Figures 2, 7). One explanation of this finding could be the decreased stiffness of Achilles tendon with severe ankle arthropathy in PWHA (Cruz-Montecinos et al. 2019b), affecting muscle force transmission to the

calcaneus. This neural adaptation may be a compensatory strategy to maintain muscle fascicles work at about constant length (Ishikawa et al. 2005; Lichtwark and Wilson 2006), causing an extra stretch of the Achilles tendon (Suydam et al. 2015). A second explanation for the altered neuromuscular control may be the newly formed connective tissues (i.e., scar tissue) between SOL and gastrocnemius (GA), as a result of repetitive intra-articular and intra-/intermuscular bleedings in PWHA (Melchiorre et al. 2017; Ribbans and Rees 1999). In a recent study on rats, a shift from preferential recruitment of SOL muscle to preferential recruitment of GA muscle during locomotion in response to increased stiffness of intermuscular connective tissues was reported (Bernabei et al. 2017). To assess this for our data, we calculated the slope ratio between SOL/LG EMG during the first half of the stance phase at the same gait velocity (Bernabei et al. 2017). The slope ratio of PWHA was lower (53.6% less, $p = 0.028$, $d = 0.90$) than that of CG, indicating a relative shift toward preferential recruitment of LG. These results are in agreement with adaptations in response to enhanced connectivity between SOL and LG (Bernabei et al. 2017). However, future studies are needed to confirm changes in the mechanical coupling between ankle plantar flexors in PWHA.

We found a relatively lower activity (~20% less) of SOL muscle observed during the push-off phase at 40% of the gait cycle in PWHA (Figure 2). A lower amplitude of SOL activity during the stance phase has also been reported in people with ankle arthrodesis (Wu et al. 2000). The relatively lower activity of SOL muscle at 40% of the gait cycle observed in PWHA may be explained by a lower muscle force (Af Klint et al. 2010; Sinkjær et al. 2000), reducing force feedback, and less dorsi-flexion of the ankle joint (Figures 2, 8), reducing length feedback.

The early onset of GA activity and the resulting greater overlap with TA activity during the stance phase (Figure 7), as well as the higher co-contraction between MG-TA and LG-TA observed in PWHA, indicate altered coordination between antagonistic muscles. In PWHA, increased co-contraction between antagonistic muscles (i.e., TA, MG, and LG) has been reported only during a static task (i.e., upright posture), which appears to compensate for their joint damage (Kurz et al. 2012, 2019). The increased co-contraction between MG-TA and LG-TA during stance, as found here, has also been reported in people with ankle OA (Doets et al. 2007; von Tscharner and Valderrabano 2010). This may be a strategy to increase the stability of the ankle joint during reception and push-off action, also limiting the load exerted on the ligaments and cartilage tissue (O'Connor 1993). Note, however, that co-contraction increases the intraarticular load (Trepczynski et al. 2018), which may accelerate the progression of cartilage degeneration (Griffin and Guilak 2005; Knarr et al. 2012; Richards and Higginson 2010).

In the muscles crossing the knee, we observed relatively higher amplitudes of activation and later offset of knee flexors and extensors during the stance phase in PWHA. Several studies have been reported that longer and relatively higher activation of knee flexors in people with knee OA contributes to joint degeneration (Hodges et al. 2016; Hubley-Kozey et al. 2009; Trepczynski et al. 2018). Different mechanisms have been proposed to explain the longer activation of knee flexors during gait in knee OA. The laxity of the knee joint could contribute to adopting a more prolonged activity of knee flexors during the acceptance phase facilitating joint stability (Hubley-Kozey et al. 2008; Lewek et al. 2004). In addition, similar to the Achilles tendon the mechanical properties of the patellar tendon may be more compliant in PWHA, contributing to a later offset of VM and VL.

The later offset in knee flexors and the resulting greater overlap with activity of knee extensors during the stance phase (Figure 7), as well as higher co-contraction between VL-BF, VM-ST, LG-VL, and MG-VM observed in PWHA, indicate altered coordination between antagonistic muscles. The higher co-contraction between knee flexor and extensor muscles has been reported in people with knee OA (Alnahdi et al. 2012; Hodges et al. 2016; Hubley-Kozey et al. 2009; Preece et al. 2016). Quadriceps strength is strongly and inversely correlated with muscle co-contraction in both healthy people and people with knees with articular cartilage defects (Thoma et al. 2016). In people with knee OA and PWHA, a reduced quadriceps strength has been reported (Alnahdi et al. 2012; González et al. 2007; Hilberg et al. 2001). These results suggest that the higher co-contraction between muscles crossing the knee may serve to stabilize the joint similar to that reported for people with knee OA (Smith et al. 2019). In PWHA, we also observed that the co-contraction between GA and knee extensors during the stance phase is increased. Similar results have been reported in people with knee OA (Lewek et al. 2005). Owing to the biarticular function of GA (i.e., plantar flexion and knee flexion), the higher co-contraction observed between GA and knee extensors in PWHA may be an adaptation to increase the stability of the knee during the stance phase, but this may also have consequences for its function at the ankle joint.

In addition to the above-described factors (e.g., muscle strength, joint stability), pain has been related to changes in neuromuscular control during gait in knee OA (Hortobágyi et al. 2005). This has received little attention in PWHA (Cruz-Montecinos et al. 2019a). The intensity of pain during gait reported in this study was mild (median of Visual Analogue Scale 1; min, 0; max, 5 points, Table 1).

Therefore, we do not expect that pain plays an important role in our results. However, futures studies are needing to assess if and in what way the intensity of pain and duration of arthropathy symptoms affects neuromuscular control during gait and more challenging activities (i.e., stair negotiation) (Smith et al. 2019).

The present results may help to design new physical therapy approaches to improve the neuromuscular control during gait in PWHA. In knee OA, for instance, it has been found that a neuromuscular re-education (Preece et al. 2016) and exercise program integrated with self- management education reduced the co-contraction between knee extensors and flexors during gait (Al-Khlaifat et al. 2016). However, future studies are needed to probe if these approaches mentioned above have the same effects in PWHA.

Changes in Leg Kinematics

We found in PWHA a reduced sagittal plane ROM in the ankle, knee, and hip. The reduced ankle ROM in PWHA compared to controls during preferred and slow velocity (mean difference 6.4 and 4.8◦, respectively), was similar than reported in previous studies in people with ankle OA and haemophilic ankle arthropathy (Lobet et al. 2010; Nüesch et al. 2014). In addition, PWHA showed a lower amplitude of plantar flexion compared to the CG during preferred velocity and lower amplitude of dorsi-flexion for the ankle joint compared to the CG during slow velocity. The lower amplitude of plantar flexion could be explained by the altered co-contraction and lower stiffness of the Achilles tendon. The decreased plantar flexion observed in PWHA may be explained by the increased co- contraction between GA and TA and reduced SOL activity during push-o similarly to that reported in elderly (Franz and Kram 2013). The lower stiffness of the Achilles tendon reported in PWHA (Cruz-

Montecinos et al. 2019b) and elderly (Delabastita et al. 2019), affecting force transmission from triceps surae muscles to the calcaneus (Don et al. 2007), maybe responsible for less ankle plantar flexion. The decreased dorsi-flexion in PWHA may be explained by articular and non-articular factors. Different surgical approaches improve dorsi-flexion (i.e., increased passive ROM) in PWHA, such as the release of the posterior joint capsule (Barg et al. 2016), the anterior osteophyte on the tibiotalar joint (Yoo et al. 2019), and Achilles tendon lengthening (Ribbans and Rees 1999).

The PWHA recruited in our study had a passive ROM higher than 60◦ in the knee. Despite that, in PWHA, a smaller knee flexion angle and lower ROM were observed compared with CG for both velocities. In knee OA, several studies have reported the impact of a limited knee ROM on gait (Al-Zahrani and Bakheit 2002; Astephen et al. 2008a; Weidenhielm and Gök 2003). The limited knee ROM compared to CG during preferred and slow velocity (mean difference, 14 and 12◦, respectively) was similar to that reported in previous studies in people with knee OA (Astephen et al. 2008a; Weidenhielm and Gök 2003). One explanation of this finding could be a lower knee flexion velocity at toe-off, resulting in a lower peak knee flexion during the swing phase (Goldberg et al. 2003, 2004; Piazza and Delp 1996). In addition, in the present study, knee flexion velocity at toe-off was lower in PWHA (27%, $p < 0.001$) than that of CG (data not shown).

In the hip joint, we observed a lower ROM in PWHA comparing to CG at the preferred velocity and lower flexion angle during the swing phase in PWHA comparing to CG at both velocities. It is not common in PWHA that the hip is affected by recurrent bleeding, and the hip is generally affected at a later stage (Carulli et al. 2017). Therefore, the lower hip flexion angle during the swing phase

may be (partly) explained by changes of knee and ankle muscle activity and the reduced step length.

Limitations

This study has some limitations. First, joint kinetics were not assessed. Therefore, it is unclear if loading of the joints, and hence the mechanical demands, were different in PWHA. Second, the applied EMG normalization method, the maximum value of all included steps, limits the interpretation of differences in intensity between groups (Benoit et al. 2003; French et al. 2015). We selected this method, and not one using EMG during maximal voluntary contraction, because the latter cannot be recorded reliably due to the potential provocation of pain incrementing the intersubject variability (Cronin et al. 2015). Third, due to the greater variability of gait observed in PWHA, an outlier may reduce the normalized EMG values for the other cycles and, thus, affect the mean. However, using the median instead of the maximum, as proposed earlier (Cheung et al. 2009), did yield similar results. Four, we used IMU sensors to assess only the sagittal plane kinematics of hip, knee, and ankle joints. The other planes (i.e., internal–external rotation and abduction–adduction) were not assessed because the accuracy is inadequate (Zhang et al. 2013). The IMU is less sensitive to detect changes than the traditional camera-based optical motion capture systems (Cooper et al. 2009). Despite that, in our study, we observed significant differences between PWHA and CG in ankle, knee, and hip joint kinematics. In addition, the kinematics of the CG were similar to those reported for healthy people during overground walking assessed with traditional camera-based optical motion capture systems (Fukuchi et al. 2018) and IMU sensor in knee OA (Chapman et al. 2019). Five, to exclude joint disease in the CG, we used the (Brinkmann and Perry 1985)HJHS and clinical criteria (i.e., Alt-man's criteria)

based on previous studies in knee OA (Altman et al. 1986; Na and Buchanan 2019). It would have been better to confirm the inexistence of asymptomatic joint disease for the CG using radiological scores by magnetic resonance imaging or ultrasound (De la Corte-Rodriguez et al. 2018; Lundin et al. 2012).

CONCLUSION

In conclusion, the neural control of individual muscles and coordination between antagonistic muscles during gait in PWHA differs substantially from control subjects. To explain the changes in neuromuscular control in PWHA, future studies should focus on the potential mechanisms, their interaction with joint damage, and possibly pain.

CHAPTER 5

Modular reorganization of gait in chronic but not in artificial knee joint constraint

Introduction: It is currently unknown if modular reorganization does occur if not the central nervous system, but the musculoskeletal system is affected**. Aim**s**:** The aims of this study were to investigate 1) the effects of an artificial knee joint constraint on the modular organization of gait in healthy subjects; and 2) the differences in modular organization between healthy subjects with an artificial knee joint constraint and people with a similar but chronic knee joint constraint. **Methods:** Eleven healthy subjects and eight people with a chronic knee joint constraint walked overground at 1 m/s. The healthy subjects also walked with a constraint limiting knee joint movement to 20 °. The total variance accounted (tVAF) for one to four synergies and modular organization were assessed using surface electromyography from 11 leg muscles. **Results:** The distribution of number of synergies were not significantly different between groups. The tVAF and the motor modules were not significantly affected by the artificial knee constraint. A higher tVAF for one and two synergies, as well as merging of motor modules were observed in the chronic knee constraint group. **Conclusions:** We conclude that in the short-term a knee constraint does not affect the modular organization of gait, but in the long-term a knee constraint results in modular reorganization. These results indicate that merging of motor modules may also occur when changes in the mechanics of the musculoskeletal system is the primary cause of the motor impairment.

Cruz-Montecinos C, Pérez-Alenda S, Cerda M, Maas H. Modular reorganization of gait in chronic but not in artificial knee joint constraint. J Neurophysiol. 2021 Aug 1;126(2):516-531. doi: 10.1152/jn.00418.2020.

INTRODUCTION

Several neurological (e.g., stroke and cerebral palsy) and musculoskeletal diseases (e.g., osteoarthritis, rheumatoid arthritis, and haemophilic arthropathy) result in a chronic limitation in joint range of motion (ROM) (Brinkmann and Perry 1985; Campbell et al. 2015; Chen et al. 2005; Cloodt et al. 2018; Fergusson et al. 2007; Solimeno et al. 2010). This interferes with many essential activities of daily living such as walking, stair climbing, or standing up (Andriacchi et al. 1980; Hemmerich et al. 2006; Rowe et al. 2000) affecting people's ability to function independently and their quality of life (Clavet et al. 2015; Leblebici et al. 2006).

The success of surgical and pharmaceutical treatments to recover joint kinematics and neuromuscular control during gait is limited (Ardestani et al. 2017; Shuman et al. 2016), especially when the knee joint is affected (Benedetti et al. 2003; Van Criekinge et al. 2020). To improve treatment and rehabilitation strategies, a more detailed analysis of the neural consequences of chronic joint constraints seems warranted.

For neurological and musculoskeletal diseases, the mechanisms causing the chronic limitation in joint ROM are different. In neurological diseases, a disruption of the central and/or peripheral nervous system is the primary cause of the joint constraint (Van Criekinge et al. 2020; Dean et al. 2020; Kaya et al. 2019; Sulzer et

al. 2010; Wang et al. 2017). In musculoskeletal disorders, the joint constraint is the result mainly of changes in bone or soft tissues (Campbell et al. 2015; Solimeno et al. 2010; Tarabichi and Tarabichi 2010; Trudel et al. 2014; Wang et al. 2019). In case a disorder of the musculoskeletal system is the primary cause, the central nervous system may also be affected. Such secondary adaptations may lead to altered neuromuscular control during gait, for example, an increased coactivation between antagonistic muscles (Cruz-Montecinos et al. 2019a, 2020c; Hubley-Kozey et al. 2006, 2009). Recently, a temporary immobilization of rat hindlimbs (16 h a day for 28 days) during development was found to cause not only a chronic restriction of joint ROM during locomotion, but also a persistent spinal hyperreflexia and changes in topographical organization of the sensorimotor cortex (Delcour et al. 2018b, 2018a).

The central nervous system is presumed to produce limb movements, not by exciting individual muscles but by exiting a group of muscles in patterns called muscle synergies (Chvatal and Ting 2013; D'Avella et al. 2003; Tresch et al. 1999, 2006). A muscle synergy can be defined as a selected group of muscles that are recruited simultaneously with distinct relative levels of activation (Bizzi et al. 2002; Bizzi and Mussa-Ivaldi 1998; Tresch et al. 1999, 2006). Muscle synergies can be characterized by electromyographic (EMG) activities of muscles represented by combinations of time-independent weights (motor modules) and time-dependent coefficients (motor primitives) (Dominici et al. 2011; Gizzi et al. 2011; Hart and Giszter 2010; Santuz et al. 2017, 2018). The motor modules and motor primitives represent together the modular organization of the movement pattern (Santuz et al. 2019).

In healthy individuals, four different muscle synergies have previously been identified to reconstruct unilateral lower extremity muscle activation patterns during locomotion, each related to a specific part of the gait cycle (e.g., acceptance, propulsion, swing, deceleration) (Clark et al. 2010; Neptune et al. 2009). Synergy 1, acceptance (with a principal contribution of hip extensors/abductors and knee extensors) is exited during early stance. Synergy 2, propulsion (with a principal contribution of ankle plantar flexors) is exited during late stance. Synergy 3, swing (with a principal contribution of ankle dorsiflexors and biarticular knee extensors) is exited during early swing. Synergy 4, deceleration (with a principal contribution of hamstring muscles) is exited during late swing. In people with chronic stroke, merging of modular organization (i.e., modular reorganization) such as acceptance and propulsion synergies or acceptance and deceleration synergies has been reported (Allen et al. 2013; Clark et al. 2010; Van Criekinge et al. 2020). However, in patients with subacute stroke (20 wk), the motor primitives were similar to those of a healthy group (Gizzi et al. 2011). It is currently unknown if such merging of modular organization does also occur when changes in the mechanics of the musculoskeletal system, not the central nervous system, is the primary cause.

In humans, the short-term effects (immediate to a few minutes following application) of artificial joint constraints have been studied using external devices. For example, to mimic the knee flexion contracture gait pattern typical for people with cerebral palsy and people with knee arthropathy and to mimic full knee extension following knee arthrodesis surgery (Attias et al. 2016, 2019; Cerny et al. 1994; Cook et al. 1997; Harato et al. 2008; Hutchison et al. 2019; Lewek et al. 2012; Perry et al. 1975; Sotelo et al. 2018). A simulated knee flexion contracture results in an increased forefoot weight bearing and flexion posture during stance, increasing the knee extensor torque (Cerny et al. 1994; Harato et al. 2008; Perry et

al. 1975). Both simulated knee flexion contracture and knee arthrodesis change ankle and hip kinematics to facilitate foot clearance of the braced limb (Attias et al. 2016, 2019; Cerny et al. 1994; Harato et al. 2008; Hutchison et al. 2019; Lewek et al. 2012). Only one previous study reported short-term changes in EMG in response to an artificial knee flexion contracture and found an increase in the amplitude and duration of the gluteus maximus, vastus lateralis, and soleus muscles during the stance (Cerny et al. 1994). It is unknown, however, if an artificial joint constraint does affect the modular control of locomotion.

In one study simulating knee arthrodesis, after the immediate adaptations, no further changes in kinematics were found within 24 h (Hutchison et al. 2019). Similar results have been reported following the application of an artificial ankle joint constraints, that is, no significant changes in EMG amplitude of plantar flexors or kinetics gait parameters between immediate and post accommodation (1 h/6 wk) (Geboers et al. 2002; Zellers et al. 2019). The above suggest that a longer duration (chronic) joint restriction is required for subsequent changes in the central nervous system. Note that an acute joint restriction may not fully mimic the condition of the musculoskeletal system of people with a chronic joint restriction, because the latter may involve additional adaptations such as muscle weakness and changes in muscle length (Hoang et al. 2014; Lieber and Fridén 2019; Segal et al. 2010; Yoshida et al. 2013).

The aim of this study was to assess short-term (immediate to a few minutes following application) and long-term (years) effects of a constraint of the knee ROM on the modular organization of gait. We hypothesize that the modular organization of gait will not be affected in the short-term, but substantially changed in the long-term. For this purpose, we assessed 1) the acute effects of an artificial knee joint

constraint on the modular organization in healthy subjects; 2) the differences in modular organization between healthy subjects with an artificial knee joint constraint and people with a similar but chronic knee joint constraint.

METHODS

Participants

This study was approved by the local ethical committee and conducted in agreement with the Declaration of Helsinki. All participants were informed about the purpose and procedures of the project and gave their written informed consent to participate in the study.

For the chronic knee constraints group, people with haemophilic arthropathy (PWHA) were recruited (Fig. 1). Haemophilia is an X chromosome-linked bleeding disorder (thus affecting males only) caused by a deficiency of coagulation factors VIII (haemophilia A) and factor IX (haemophilia B) (Oldenburg et al. 2004). Repetitive intra-articular and/or intermuscular bleeding is a common manifestation of this disease (Pulles et al. 2017), which results in inflamed synovium and irreversible change in cartilage tissue, which may be accompanied by excessive fibrous or scar tissue within a joint and the consequent restriction of ROM (Solimeno et al. 2010).

Eight PWHA and eleven healthy control subjects (CG) were recruited. For the CG, inclusion criteria were the following: male, over 18 yr of age and under 65 yr, no haemophilia, and body mass index lower than 30. Exclusion criteria were the following: traumatic injuries; signs or symptoms of injury or symptomatic arthritis

to the trunk, lower back, and lower limb within the past 3 mo; scoliosis; history of cardiac and/or respiratory pathology; and neurological disease.

For PWHA, inclusion criteria were the following: diagnosed with haemophilia A or B, severe or moderate, diagnosed by a medical doctor specialized in haemophilic arthropathy, less than 30 ° of knee ROM [note that 30 ° corresponds to 50% of the knee ROM during gait in healthy individuals (Winiarski et al. 2019)], over 18 yr of age and under 65 yr, prophylaxis treatment with deficient factor(i.e., VIII or IX), and body mass index less than 30. Exclusion criteria: history of hip, knee or ankle arthroplasty, equinus foot, inability to walk without an assistive device (e. g., walker, cane), history of muscle or joint bleeding in lower limbs in the last 2 mo, chronic cardiac and/or respiratory pathology and neurological disease.

Figure 1. *A*: subject with an articulated knee brace (Blunding, Santiago, Chile) used to acutely constrain the knee range of motion to 20^0–40^0. *B*: patient with a chronic (10 yr) knee joint constraint.

Clinical Assessments for PWHA

To assess the intensity of pain (scale 0–10 points) during barefoot walking, the Visual Analogue Scale was used. The joint health status in the knee and ankle was assessed with the Haemophilia Joint Health Score 2.1 (HJHS) (Gouw et al. 2019). This scale (range 0–20) consists of eight items per joint, evaluating 1) joint swelling (0–3 points), 2) duration of swelling (0–1 points), 3) muscle atrophy (0–2 points), 4) strength (0–4 points), 5) crepitus on motion(0–2 points), 6) flexion loss (0–3 points),7) extension loss (0–3 points), and 8) pain (0–2 points). For the passive knee ROM assessment, a 360° universal plastic goniometer with a 30-cm movable arm and scale of 1° increment (Baseline, Chattanooga Group Inc.) was used for all measurements. The protocol was based on a recommendation of the measurement of passive ROM in patients with knee restrictions (Brosseau et al. 2001).

Surface Electromyography Protocol

Surface electromyography (EMG) was collected from gluteus maximus (GMAX), gluteus medius (GMED), biceps femoris (BF), semitendinosus (ST), rectus femoris (RF), vastus medialis (VM), vastus lateralis (VL), tibialis anterior (TA), soleus (SOL), lateral gastrocnemius (LG), and medial gastrocnemius (MG). In PWHA, the limb with the lowest knee ROM was selected considering the inclusion criteria. In CG, the dominant limb was assessed. Leg dominance was assessed by asking the subjects which leg they would use to kick a ball (Cruz-Montecinos et al. 2019a). After shaving and cleaning the skin with alcohol, surface electrodes (Ag–AgCl, Kendall H124SG) were placed (interelectrode spacing 2 cm) on the earlier described muscles according to SENIAM guidelines (Hermens et al. 2000). The EMG signals were measured using a wireless EMG system (MyoSystem DTS, Noraxon USA Inc.,

Scottsdale, CA) with a sampling rate of 1,500 Hz. Heel strike was detected by a synchronized wireless pressure sensor (Noraxon USA Inc., Scottsdale, CA,) placed underneath the heel of the foot.

Kinematics Protocol

The sagittal kinematics of the hip, knee, and ankle joints were assessed using on inertial measurement units (IMUs). Four IMU sensors (Xsens, Enschede, the Netherlands) were positioned between posterior superior iliac spines, the lateral face of the thigh within the proximal third, lateral face of the shank within the distal third close to the lateral malleolus, and the midfoot. The sensors placed on sacrum, thigh, and shank were fixed with the fixation system provided by the company (Xsens, Enschede, the Netherlands). The sensor placed on midfoot was fixed by adhesive elastic taping (Leukotape K, BSN Medical, Hamburg, Germany) with sufficient tension to avoid movement artifacts. In addition, the magnetometer data for each IMUs were collected to adjusts the sagittal joint position in the upright position (see Kinematics of the lower limb section). Data were collected at a sampling frequency of 75 Hz. The IMUs and EMG/pressure sensor were synchronized with a trigger pulse.

Experimental Procedures

Surface electromyography (EMG), joint kinematics and temporal gait parameters were obtained during 30 m of bare-foot walking overground twice, at the preferred velocity for PWHA and for CG at a slower velocity (1 m/s). For both EMG and kinematics, ten cycles corresponding to the middle 10 m of the 30-m corridor were used for data analysis (20 cycles in total) (Cruz-Montecinos et al. 2019a; Tang et al. 2015)(Fig. 2).

For the CG, the slow velocity was practiced three times for 10 m. Then, CG was invited to walk 30m at 1 m/s twice used an articulated knee brace (Blunding, Santiago, Chile) fixed at 20° of flexion to 40° of flexion (Fig. 1). Each participant practiced with the external device three times for 10m.

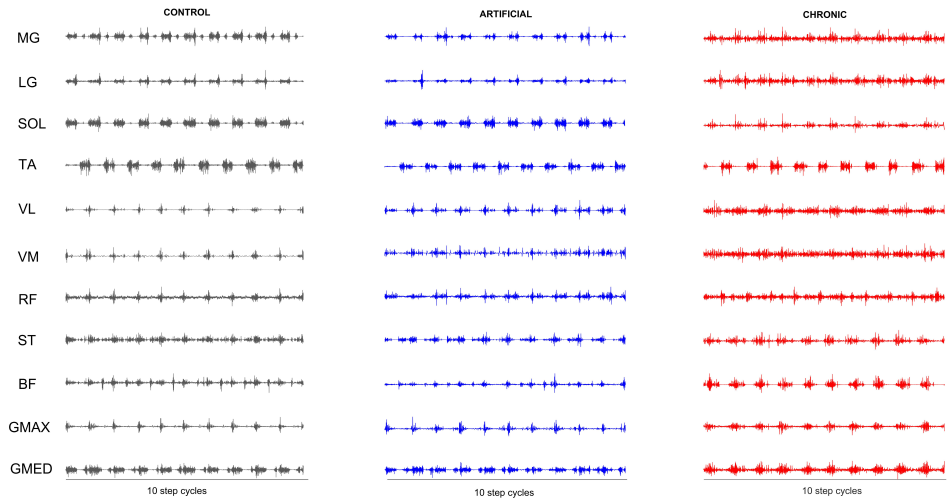


Figure 2. Surface electromyography recordings of one participant in each group including 10 cycles selected of the middle 10 m of one test of 30-m walking. BF, biceps femoris; GMAX, gluteus maximus; GMED, gluteus medius; LG, lateral gastrocnemius; MG, medial gastrocnemius; RF, rectus femoris; SOL, soleus; ST, semitendinosus; TA, tibialis anterior; VM, vastus medialis; VL, vastus lateralis.

Data Analysis

For EMG and kinematic analysis, MATLAB v. 2019a was used (Statistics and Machine Learning Toolbox, MathWorks, Inc., Natick, MA).

Muscle activity patterns and timing of EMG activity.

To assess the EMG amplitude for each muscle, first a bandpass filter (20–500 Hz, Butterworth, fifth-order) was applied. Then the EMG signals were rectified using the absolute values of Hilbert transform using the MATLAB function "Hilbert" (Pijnappels et al. 2006), and then smoothed with a low-pass filter at 10 Hz (Cappellini et al. 2006; Cruz-Montecinos et al. 2019a; Martino et al. 2015). The EMG signals during each step cycle (from heel strike to heel strike) were time normalized to 200 points, and the amplitude for each muscle was normalized to the maximum activation of each muscle recorded for each participant at each condition (Clark et al. 2010; Janshen et al. 2020; Steele et al. 2015).

Computation of muscle synergies.

The non-negative matrix factorization (NNMF) was used to extract muscle synergies from the EMG data combined into an m × t matrix (220 × 200), where m represents the number of muscles (11 and 20 cycles), and t is the time base (200 points). The gait cycles were concatenated in columns on the assumption of time-varying doses (the temporal synergy model) (Cheung and Seki 2021; Turpin et al. 2021). The NNMF results in the muscle weightings for each synergy (W) and the matrix encoding the activation pattern of each synergy (C) (Tresch et al. 2006). Note that the product of W and C should approximate the original EMG data. The NNMF algorithm was iterated 20 times for each number of synergies (Cheung et al. 2020) (from 1 to 4 in this study), and the iteration with the lowest reconstruction error was selected. To obtain the 11-element modules, averaging across 20 cycles was applied. The difference between the reconstruction of EMG data (EMGr) and the original EMG (EMGo) data was calculated using total EMG variance accounted for

(tVAF). The tVAF was calculated as the ratio of the sum of the squared error values to the sum of the squared EMGo (Clark et al. 2010), as represented in Eq. 1:

$$tVAF = 1 - \frac{\sum_{i=1}^{m}\sum_{j=1}^{t}\left(EMGo\ (i,j) - EMGr\ (i,j)\right)^2}{\sum_{i=1}^{m}\sum_{j=1}^{t}\left(EMGo\ (i,j)\right)^2}$$

Complexity of motor control.

To assess the complexity of motor control during gait, the following parameters were calculated: tVAF by one to four muscle synergies, and the lowest number of muscles synergies for tVAF > 90% (first criterion) while adding an additional synergy did not increase tVAF by > 5% (second criterion) (Clark et al. 2010). For all groups, application of the second criterion resulted in an increase of the number of synergies to the same extent (i.e., >90% of the subjects). A higher tVAF indicate an increase of coactivation between antagonistic and synergistic muscles (Cruz-Montecinos et al. 2019a; Dewolf et al. 2020; Steele et al. 2015).

Motor Modules and Motor Primitives

To allow for comparison of motor primitives and motor module between groups, we further investigated the four-synergy solution for all individuals. This number was motivated by the fact that most previous studies identified four muscle synergies during walking using the same muscles used in the present study (Clark et al. 2010; Neptune et al. 2009).

Because extraction of muscle synergies with NMMF (4 in our case) does not extract the synergies in the same order for each individual, the following methodology was applied to allocate the motor modules and motor primitives to each of the four synergies. First, we identified the best match between the synergies and the average of the four synergies extracted in the control group using the threshold of tVAF > 90% (Fig. A1). In an iterative approach, we compared the synergies in two sets by computing the cosine similarity between the best matched pairs of motor modules (Booth et al. 2019). Second, to ensure the correct allocation of each motor module to their respective synergy, the matched synergies were care-fully checked by a visual inspection focused on the muscle weightings in the motor modules and the temporal peaks of the motor primitives. Finally, each motor primitive was normalized by the peak activity and each motor module was normalized to the maximal muscle weighting.

To assess the principal muscle weighting in each module, 95% of the confidence interval was used (Hayes et al. 2014). To assess the similarities of motor modules and motor primitives between groups, the cosine similarity was computed. Where a similarity of 1 represents perfect similarity, and a value above 0.8 was defined as similar (Booth et al. 2019; Oliveira et al. 2014).

Assessment of synergy merging.

To assess if synergies were merged, we modeled the motor modules of the artificial and chronic constraint groups as a linear combination of motor modules from the control group. For the linear combinations of motor modules and their relative contribution to the merged module, the nonnegative least squares implemented with the “isqnonneg” option in MATLAB was used (Cheung et al. 2012). To assess if

a synergy of the artificial or chronic constraint groups may be reconstructed from a pair of merged synergies of the control group, two criteria were adopted. First, the coefficient of the reconstruction (i.e., contribution coefficient) of both pairs of motor modules for control group had to be above of 0.3 (Barroso et al. 2014). Second, a similarity between the reconstructed and the single motor module had to be above 0.8 (Booth et al. 2019; Hayes et al. 2014; Oliveira et al. 2014). In addition, the frequency of appearance of merged synergies according to the above criteria was calculated.

Kinematics of the lower limb.

The magnetometer data were used to determine the initial position of the hip, knee, and ankle joint in the sagittal plane. To assess the position of each joint, each subject was invited to stand in a neutral position with both feet parallel for 10 s. Then, the flexion-extension angle for the hip, knee, and ankle was estimated using the IMU quaternion orientation. Low-pass Butterworth filters were applied to the IMU joint angle data, with a cut-off frequency of 10 Hz. The segmentation of kinematic signals into strides were determined through gait events signifying heel strike and toe-off from the angular velocity in z-axis obtained from the IMU sensor of the shank (De Vroey et al. 2018b) and then time normalized to 75 points. To assess the walking velocity in the 30-m walking test, the start and end of walking was assessed using the acceleration signal of the IMU sensor of the shank. For the final analysis, the mean of the same 20 cycles selected for the EMG analysis were used to represent the kinematics and temporal parameters of gait.

Statistics

Sample size calculation.

The a priori power analysis conducted in G-Power (3.1.9.2 v.) software (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) showed that eight subjects per group were sufficient to obtain a statistical power of 0.80 with an a =0.05 and large effect size (Cohen's d = 1.65) based on the difference in walk-DMC index between CG and PWHA with severe joint damage reported previously (Cruz-Montecinos et al. 2019a).

Statistical analysis.

Two independent assessments were made for all variables: 1) comparison between CG no constraint (control) and artificial knee joint constraint (artificial) and 2) between artificial joint constraint and PWHA with chronic knee constraint (chronic constraint). The normality of data was evaluated through the Shapiro–Wilk test. For all comparisons, the α level was set at 0.05. The unpaired or paired two-tailed t test, and two-tailed Wilcoxon rank-sum test (unpaired) or Wilcoxon signed-rank (paired) were used to compare the between-subject (artificial vs. chronic) and within-subject differences (control vs. artificial), depending on the normality of data. To compare the distribution of the number of synergies, and the frequency of merged synergies between groups the Chi squared test (χ^2) was applied (Clark et al. 2010; Cruz-Montecinos et al. 2019a). To test for differences in the frequency of merged synergies between the artificial and chronic constraint groups, a post hoc test with Bonferroni correction was applied. The data are expressed as mean and standard deviation unless stated otherwise.

To evaluate differences in the tVAF as a function of number of synergies, a two-way ANOVA (within subject factor: number of synergies; between subject factor: group) was used. Greenhouse–Geisser correction was used if the assumption of sphericity, as checked by Mauchly'stest, was violated. If a significant interaction was found between factors, post hoc tests with Bonferroni correction were applied.

To test for the differences between groups in the contribution of individual muscles to each motor module, the Bonferroni correction of the P values for the total number of comparisons (i.e., 44) was applied. To compare the waveforms of the motor primitives and kinematics of hip, knee, and ankle joints between groups, n dimensional statistical (non) parametric mapping (SPM) was used (Ismailidis et al. 2020; Mehryar et al. 2020; Mileti et al. 2020; Pataky 2010). For that, the open-source MATLAB-based spm1d-package was used to generate the two-tailed t values maps (http://www.spm1d.org/index.html). Normal distribution of data was tested for the selection of parametric or nonparametric analysis. The motor primitives and joint kinematics time series were considered significantly different if any values of SPM over the entire gait cycle exceeded the critical threshold (a =0.05). To compare the motor primitives between groups, a Bonferroni correction for the four comparisons (i.e., for each of the four synergies) was applied. To compare the joint angle patterns, a Bonferroni correction for the three comparisons (i.e., for each of the three joints) was applied. In the final step, cluster specific P values were calculated over the entire gait cycle.

RESULTS

We did not find differences in age, height, and BMI between the CG and the PWHA (chronic constraint), except for pain (Table 1). In PWHA, the joint health score

(HJHS) for knee and ankle joint was not different (P =0.160). The mean of passive ROM was 12.5° ±10° for the knee and 23.2° ±12.4° for the ankle joint (Table 2). The motor modules and motor primitives data were not normally distributed.

Table 1. Characteristics of control group and people with chronic knee restriction due to haemophilic arthropathy

Clinical Characteristic	CG (*n* = 11)	PWHA (*n* = 8)	*P* Value
Age, yr	34.6 ± 10	39.1 ± 16	0.448
Height, cm	174 ± 5	170 ± 3	0.069
Body mass index	25.1 ± 2	26.4 ± 4	0.342
Pain during walking, VAS 0–10	0 [0 0]	4.0 [1 8]	**<0.001**
Chronicity of knee joint restriction, yr	NA	15 [10 20]	NA

Parametric distribution: means ± SD; nonparametric distribution: median [range]. CG, control group; NA, not applicable; PWHA, people with haemophilic arthropathy; VAS, visual analogue scale. Significant differences ($P < 0.05$) are in bold.

Table 2. Clinical characteristics of people with chronic knee restriction due to haemophilic arthropathy

Joint Clinical Assessment	PWHA (*n* = 8)
HJHS knee score, 0–20 pts	12.6 ± 3.1
HJHS ankle score, 0–20 pts	9.9 ± 2.5
Passive knee extension, degrees	-25.0 ± 11.3
Total passive range of motion of knee, degrees	12.5 ± 10
Passive ankle dorsi flexion, degrees	9.0 ± 5.3
Total passive range of motion of ankle, degrees	23.2 ± 12.4

Parametric distribution: means ± SD. HJHS, haemophilia joint health score; PWHA, people with haemophilic arthropathy.

Effects of Artificial Knee Joint Constraint on Lower Limb Kinematics

Applying the knee constraint, the amount of hip flexion was similar during the stance phase and during the swing phase. At the knee joint, the knee constraint resulted in a more flexed position during the stance ($P < 0.001$) and more flexion angle during late swing ($P=0.015$) (Fig. 3). At the ankle joint, the knee constraint resulted in a more dorsi-flexed position during the early stance ($P = 0.013$), middle stance ($P < 0.001$), and swing phases ($P=0.001$) (Fig. 3).

The knee constraint did not affect the hip ROM (normal: 31.9° ± 6.5°; artificial: 36.3° ± 6.5°; $P = 0.203$) and the ankle ROM (normal: 24.5° ±3.6°; artificial: 21.1° ± 5.4°; $P =0.203$) during gait. A significant difference was observed for the knee ROM (normal: 49.8 ° ± 5.3°; artificial: 24.4° ± 5.6°; $P < 0.001$).

Differences Kinematics of the Lower Limb between Artificial and Chronic Constraint

The extent of knee flexion was lower in the PWHA (chronic constraint) than in the CG with the artificial constraint during the swing phase ($P =0.015$) (Fig. 3). No differences between groups were found for the hip and ankle joints (Fig. 3).

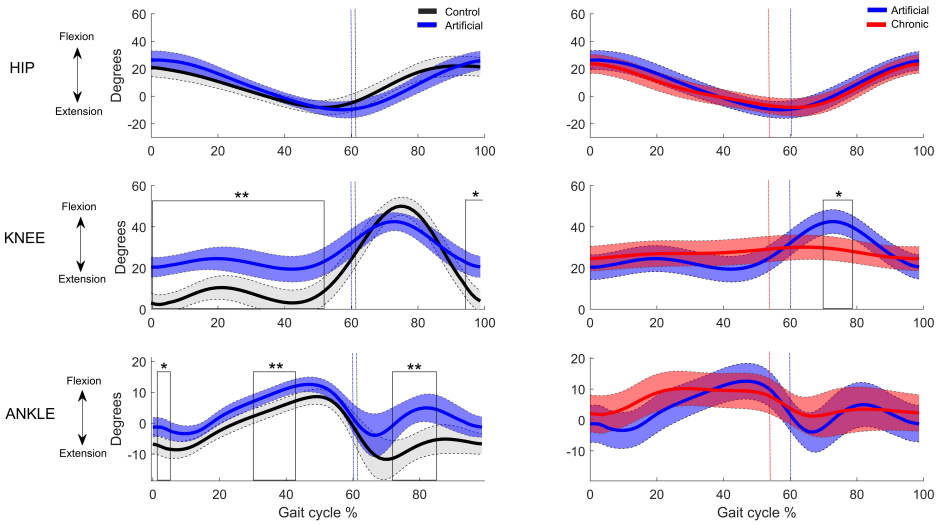


Figure 3. Joint kinematics during gait for hip (*top*), knee (*middle*), and ankle (*bottom*). Statistical results of comparisons between normal and artificial knee constraint in the healthy group (n = 11) and between an artificial (n = 11) and chronic knee constraint (n = 8) are shown. Data are plotted as a function of normalized step time (0, heel strike) and expressed as mean with 95% confidence intervals. The time series for joint kinematics were analysed with Statistical Parametric Mapping. The vertical dotted line indicates the end of the stance phase. $^{*}P < 0.05$. $^{**}P < 0.001$.

The knee (chronic: 6.3° ± 3.8°; artificial: 24.4° ±5.6°; P < 0.001) and ankle ROM (chronic: 11.6° ±4.3°; artificial: 21.1° ±5.4; P = 0.001) were lower in the PWHA (chronic constraint) than in the CG with the artificial constraint. No difference between groups was observed in the hip ROM (chronic: 33.3° ±8.3°; artificial: 36.3° ±6.5°; P =0.395).

Effects of Artificial Knee Joint Constraint on Temporal Variables

Applying the knee constraint did not result in changes of step cycle time (normal:1.2s ± 0.1 s; artificial: 1.2 ± 0.1 s; P = 0.204), stance time (normal: 61.4% ±

4.1%; artificial: 60.0% ± 1.4%; P = 0.162), and gait velocity in 30 meters walking task (normal: 1.0 ± 0.2 m/s; artificial: 1.0 ± 0.2 m/s; P = 0.655).

Temporal Variables between Artificial and Chronic Knee Constraint

The stance time was lower in the PWHA (chronic constraint) than in the CG with the artificial constraint (chronic: 54.0% ± 4.4%; artificial: 60.0% ± 1.4%; P = 0.007). No differences between groups were observed in the step cycle time (chronic: 1.1 ± 0.1 s; artificial: 1.2 ± 0.1 s; P = 0.076) and in gait velocity in 30 m walking task (artificial: 1.0 ± 0.2 m/s; chronic: 0.9 ± 0.1 m/s; P = 0.425).

Effects of the Artificial Knee Joint Constraint on Complexity of Neuromuscular Control

The distribution of muscle synergies extracted for normal walking [median 4 (min 2 max 4)] and walking with the artificial constraint [median 4 (min 3 max 4)] did not differ (P = 0.264) (Fig. 4 and Figs. A1 and A2).

Also, no differences between groups were found for the quality of reconstruction based on the number of synergies extracted (control: tVAF 95.2% ± 2.4%; artificial: tVAF 95.8% ± 1.6%; P = 0.572). The two-way ANOVA indicated a significant main effect of number of synergies (P < 0.001), but no significant group effect (P = 0.558) and no significant interaction between factors (P = 0.137) on the tVAF (Fig. 4).

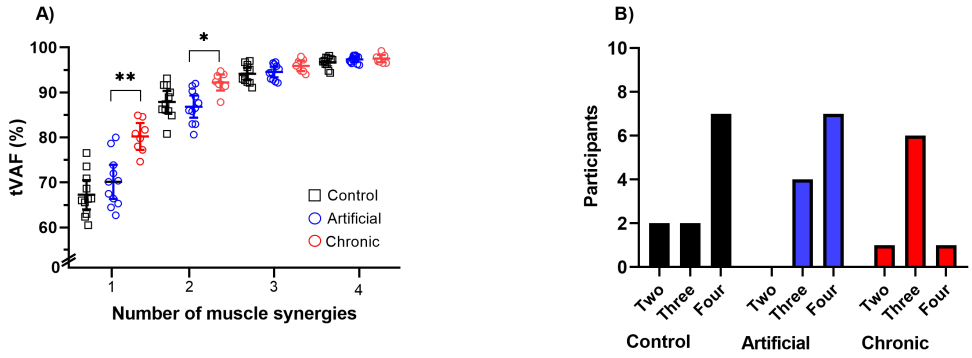


Figure 4. A: the total variance accounted for (tVAF) by 1–4 muscle synergies. Statistical results of comparisons between no and an artificial knee constraint in the control group (n = 11) and between an artificial constraint in the control group (n = 11) and the chronic knee constraint group (n =8) are indicated. *P < 0.05; **P < 0.001. Individual data, means, and 95%confidence intervals are shown. **B:** the distribution of the number of muscles synergies for each group

Differences in Complexity of Neuromuscular Control between Artificial and Chronic Constraint

The distribution of muscle synergies extracted for the chronic constraint group [median 3 (min 2 max 4)] was not statistically different from that of the artificial knee constraint group (P =0.062) (Figs. 4, 5, and A2). Also the quality of reconstruction based on the number of synergies extracted was not statistically different between groups (chronic: tVAF 95.6% ± 2.0%; artificial: tVAF 95.8% ± 1.6%; P = 0.838). The two-way ANOVA indicated a significant main effect of number of synergies (P < 0.001) and significant main effect of factor group (P = 0.001), as well a significant interaction between factors (P < 0.001) on the tVAF. Post hoc analysis

showed a significant difference between groups when including one synergy ($P < 0.001$) and two synergies ($P = 0.002$) (Fig. 4).

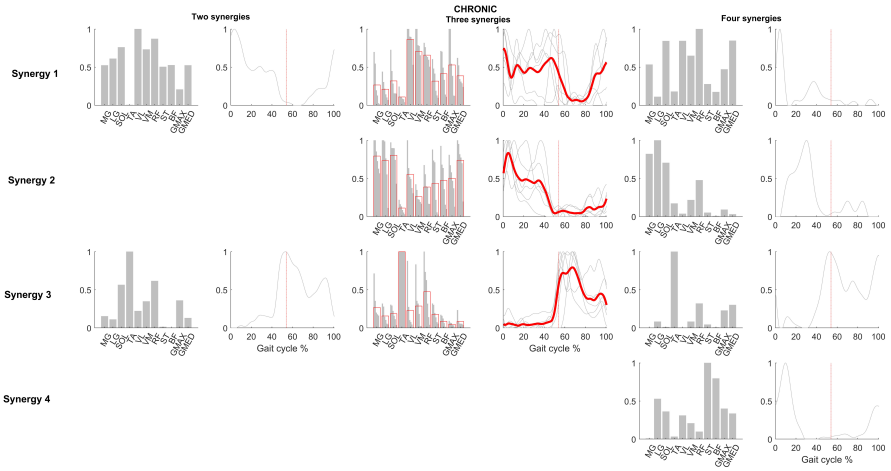


Figure 5. The average and individual data for motor modules and motor primitives of chronic constraint based on the tVAF > 90% criterion. The motor primitives are plotted as a function of normalized step time (0, heel strike). The vertical dotted lines indicate the end of the stance phase. a.u, Arbitrary units; BF, biceps femoris; GMAX, gluteus maximus; GMED, gluteus medius; LG, lateral gastrocnemius; MG, medial gastrocnemius; RF, rectus femoris; SOL, soleus; ST, semitendinosus; TA, tibialis anterior; tVAF, total variance accounted for; VM, vastus medialis; VL, vastus lateralis.

Effects of Artificial Knee Joint Constraint on Modular Organization

Applying the knee constraint did not result in statistically different individual muscle contributions (i.e., motor modules) (Figs. 6 and 7). The similarity of motor modules between groups was higher than 0.8 for all synergies (Table 3). Different

patterns of the motor primitives between groups were found for synergy 1, 2 and 3. For synergy 1 (acceptance), the motor primitives of the artificial constraint group show a higher activity during the late swing phase (~80% of the step cycle; $P < 0.001$) (Fig. 7). For synergy 2 (propulsion), the motor primitives of the artificial constraint group show a lower activity during the late stance phase (~40% of the step cycle; $P < 0.001$) (Fig. 7). For synergy 3 (swing), the motor primitives of the artificial constraint group show a lower activity during the late swing phase (~80% of the step cycle; $P < 0.001$) (Fig. 7). The similarity of motor primitives between groups was lower than 0.8 for synergies 3 and 4 (Table 3). No merging of synergies was found in response to the artificial knee constraint (Table A1).

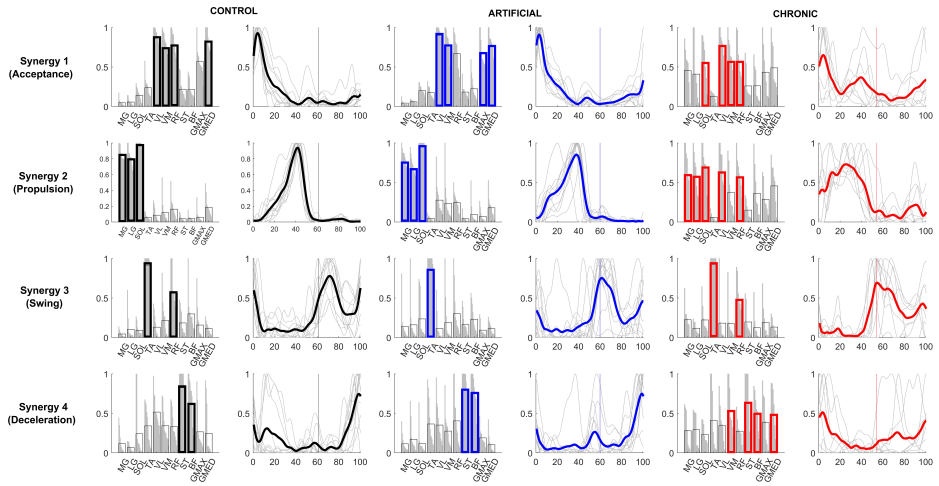


Figure 6. The average and individual data for motor modules and motor primitives for each group. In the motor modules, the bars with a thick line indicate the principal muscle contributions. The motor primitives are plotted as a function of normalized step time (0, heel strike). The vertical dotted lines indicate the end of the stance phase. a.u, Arbitrary units; BF, biceps femoris; GMAX, gluteus maximus; GMED, gluteus medius; LG, lateral gastrocnemius; MG, medial gastrocnemius; RF, rectus femoris; SOL, soleus; ST, semitendinosus; TA, tibialis anterior; VM, vastus medialis; VL, vastus lateralis.

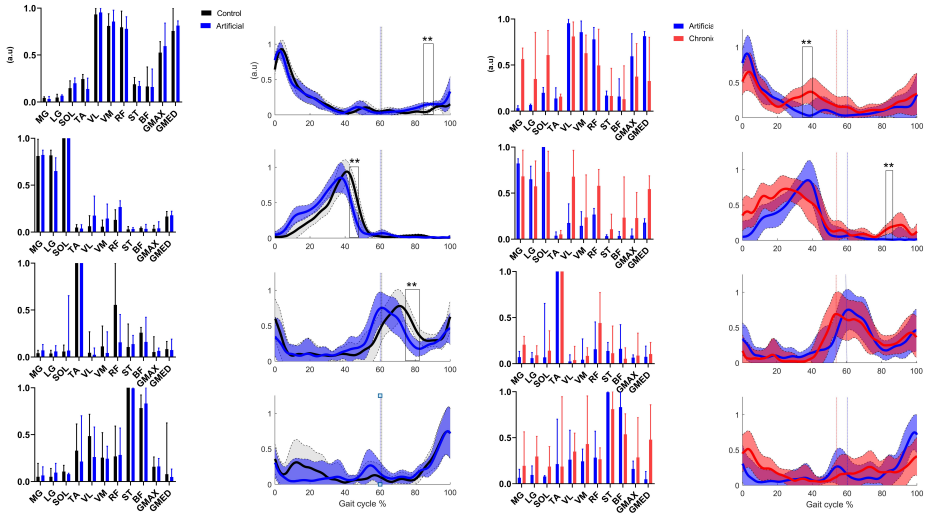


Figure 7. Comparison of motor modules and motor primitives between groups. The motor modules are shown as median and interquartile range. Motor primitives are plotted as a function of normalized step time (0, heel strike) and expressed as means with 95% confidence intervals. The time series for motor primitives were analysed with Statistical Parametric Mapping. The vertical dotted line indicates the end of the stance phase. a.u, Arbitrary units; BF, biceps femoris; GMAX, gluteus maximus; GMED, gluteus medius; LG, lateral gastrocnemius; MG, medial gastrocnemius; RF, rectus femoris; SOL, soleus; ST, semitendinosus; TA, tibialis anterior; VM, vastus medialis; VL, vastus lateralis. $^{**}P < 0.001$.

Modular Organization between Artificial and Chronic Constraint

Comparing the artificial and chronic knee constraint groups did not result in statistical differences in individual muscle contributions (i.e., motor modules) (Figs. 6 and 7). The similarity of motor modules between groups was lower than 0.8 for synergies 1, 2 and 4 (Table 3). Different patterns of the motor primitives between groups were found for synergy 1 and synergy 2 (Figs. 6 and 7). For synergy 1 (acceptance), the motor primitive of the chronic group shows a higher activity during the late stance phase (~40% of the step cycle; $P < 0.001$) (Fig. 7). For synergy

2 (propulsion), the motor primitive of the chronic group shows a higher activity during the late swing phase (~80% of step cycle; P < 0.001) (Fig. 7). The similarity of motor primitives between groups was lower than 0.8 for synergies 1 and 4 (Table 3).

Table 3. Similarity of motor modules and motor primitives between groups

Synergy	Modules Motor		Motor Primitives	
	Control (n=11) vs. Artificial (n=11)	**Artificial (n=11) vs. Chronic (n=8)**	**Control (n=11) vs. Artificial (n=11)**	**Artificial (n=11) vs. Chronic (n=8)**
(S1) Acceptance	0.96 [0.92 0.99]	**0.79 [0.66 0.91]**	0.92 [0.87 0.97]	**0.74 [0.54 0.95]**
(S2) Propulsion	0.92 [0.81 0.99]	**0.76 [0.59 0.93]**	0.86 [0.76 0.96]	0.82 [0.73 0.89]
(S3) Swing	0.81 [0.69 0.94]	0.88 [0.79 0.97]	**0.75 [0.61 0.90]**	0.81 [0.70 0.92]
(S4) Deceleration	0.86 [0.77 0.94]	**0.71 [0.59 0.84]**	**0.77 [0.64 0.90]**	**0.50 [0.33 0.67]**

Means and 95% confidence interval of cosine similarity. Similarities <0.8 are in bold. S, synergy.

A reorganization of motor modules was observed in the chronic constraint group. Synergy 1 appeared to be com-posed of synergy 1 and synergy 2 of the control group, and also synergy 2 appeared to be composed of synergy 1 and synergy 2 of the control group (Fig. 8 and Table A1). The frequency of appearance of merged synergies for synergy 1 and the frequency of appearance of merged synergies for synergy 2 were statistically different between groups (chronic 63%vs. artificial 0%, P = 0.003; chronic 50% vs. artificial 0%, P = 0.020, respectively) (Fig. 8 and Table A1). The aforementioned results indicate that a chronic constraint resulted in a modular reorganization characterized by merging of acceptance with propulsion synergies.

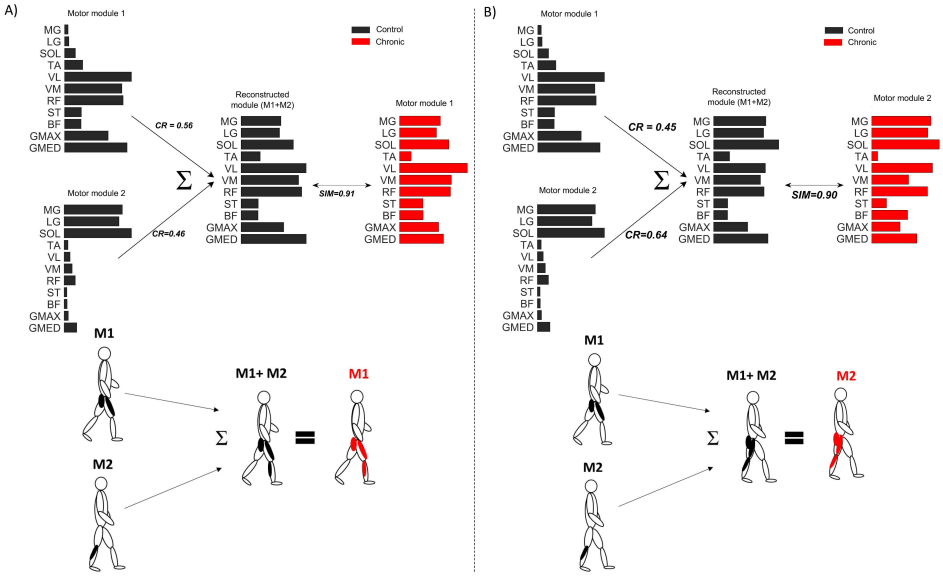


Figure 8. A reorganization of motor modules was observed in the chronic constraint group. A: synergy 1 of chronic constraint group (red) appeared to be composed of synergy 1 (acceptance) and synergy 2 (propulsion) of the control group (black). B: synergy 2 of chronic constraint group (red) appeared to be composed of synergy 1 and synergy 2 of the control group (black). For both synergies, the two criteria to determine if one synergy may be reconstructed from a pair of merged synergies were achieved. First, the coefficient of the reconstruction (CR) of the two pairs of motor modules was above of 0.3. Second, a similarity (SIM) between the reconstructed and the original motor module was above 0.8. For both synergies, the frequency of appearance was significantly different between chronic and artificial groups (Table A1). BF, biceps femoris; GMAX, gluteus maximus; GMED, gluteus medius; LG, lateral gastrocnemius; M, motor module; MG, medial gastrocnemius; RF, rectus femoris; SOL, soleus; ST, semitendinosus; TA, tibialis anterior; VM, vastus medialis; VL, vastus lateralis.

DISCUSION

The aims of the present study were to assess short-term (immediate to a few minutes following application) and long-term (years) effects of a constraint of the knee ROM on the modular organization of gait. The main results of our study are as follows: 1) similar distribution of synergies were found between groups. 2) The tVAF and modular organization of gait was not affected by the artificial knee constraint (short-term), but the timing of motor primitives of the acceptance, propulsion and swing synergies was affected. 3)For the chronic constraint, a higher tVAF for one and two synergies, and significant differences in the motor primitives for acceptance and propulsion synergies were observed. Furthermore, evidence of merged synergies in the chronic constrained group was found. These results support our hypothesis that the modular organization of gait will not be affected in the short-term, but changes following a long-term constraint of the knee joint ROM. To the authors' current knowledge, this study is the first to report the effects of artificial and chronic knee joint constraints on the modular organization of gait.

Constraining the knee joint in the control group did not result in changes of modular organization of gait, but in changes of the timing of motor primitives of the acceptance, propulsion and swing synergies. Our results are in agreement with previous results describing adaptations of the central nervous system and EMG patterns of individual muscles in response to short-term (minutes) application of an artificial knee constraint, which increased the amplitude and duration of the knee extensors and plantar flexors during the stance phase of gait (Cerny et al. 1994). Whether this involved also a reorganization of modular control was not investigated. The absence of an effect of the artificial knee constraint on the organization of motor modules may be explained by the limited time of exposure

(immediate to minutes), which may not be sufficient to recalibrate the sensorimotor system to a novel walking situation (Iturralde and Torres-Oviedo 2019; Zeni and Higginson 2010). During split-belt walking, it has been reported that the central nervous system requires a longer exposure (~15 min, 900 strides) to change neuromuscular control (Iturralde and Torres-Oviedo 2019). On the other hand, the activity of plantar flexors immediately following the application of an artificial ankle joint constraint did not differ from that after 1–6h (Geboers et al. 2002; Zellers et al. 2019). This suggests that adaptations of the central nervous system to changes in the external conditions (i.e., belt speed on treadmill) differ from those applied directly to the body (i.e., joint constraint).

Despite the fact that the artificial knee joint constraint resulted in similar ankle, knee, and hip joint kinematics (Fig. 3), modular reorganization (merging of muscle synergies) was found only in the people with the chronic constraint. Up to now, such merging of synergies has been reported only in people with neurological diseases (i.e., chronic stroke, cerebral palsy) (Allen et al. 2013; Cheung et al. 2012; Clark et al. 2010; Van Criekinge et al. 2020; Tang et al. 2015). Merging between acceptance and propulsion synergies may be explained by different factors. First, merging of these synergies and, thereby, increasing the extent of coactivation, may serve to improve the stance limb stability and decrease pain sensation during stance (Cruz-Montecinos et al. 2020c, 2021; Hubley-Kozey et al. 2009; Mills et al. 2013b; Rutherford et al. 2013). Several studies on PWHA have reported a reduced postural stability during bipedal, unipedal, and transition tasks (Cruz-Montecinos et al. 2020a; Deschamps et al. 2018; Gallach et al. 2008). In addition, the chronic group experienced pain during walking (Table 1). Joint and muscle pain is also known to affect the neural control of individual muscles (Astephen Wilson et al. 2011), and how much a muscle is involved in a motor module (Geri et al. 2019). Second, the

long exposure to the joint constraint may also result in secondary changes in the central nervous system (e.g., muscle hyperreflexia, altered somatosensory feedback, and intersegmental facilitative pathways at spinal level) (Delcour et al. 2018a, 2018b; Dyer et al. 2014), contributing to abnormal timing of leg extensors during the stance phase (Dyer et al. 2014) Third, the repetitive intra- and intermuscular bleeding and, hence, scar tissue formation is a common manifestation of haemophilia (De La Corte-Rodriguez and Rodriguez-Merchan 2014). This may change the extent of mechanical connectivity between neighboring, synergistic muscles and, thereby, their intermuscular coordination during locomotion (Bernabei et al. 2017). Future studies are needed to investigate if this also results in modular reorganization.

In the chronic constraint group, we also observed a change in the timing of the motor primitives, specifically, a prolonged activity in the stance phase (increase activity ~40% of the step cycle) of the acceptance synergy. Such prolonged activity has also been reported in children and adults people with cerebral palsy and people with chronic stroke (Booth et al. 2019; Clark et al. 2010; Yu et al. 2019). Although joint kinetics was not assessed, it is likely that also in the present study a similar increased mechanical demand for the knee extensors was present. The longer activity of the acceptance synergy may serve to compensate for the reduced ability to absorb the impact of foot placement due by the reduced knee extension (Lewek et al. 2012), and increase the stability of the stance limb during propulsion (Jonkers et al. 2003; Liu et al. 2006; Thompson et al. 2013).

The finding that modular reorganization occurred only after long-term exposure to a joint constraint may be explained by secondary changes in musculoskeletal and the central nervous systems. It has been shown that neuromuscular adaptions in

response to a joint constraint do not necessarily occur acutely (Barroso et al. 2019). The long-term exposure to a joint constraint causes secondary changes in the musculoskeletal system (e.g., joint capsule, muscle and connective properties) (Kaneguchi et al. 2017; Onoda et al. 2014; Solimeno et al. 2010; Tarabichi and Tarabichi 2010), and secondary changes in the central nervous system (Delcour et al. 2018a, 2018b).

Finally, the differences observed between acute and chronic knee constraint may not only be attributed to the knee joint restriction. PWHA are commonly affected by arthropathy at both the knee and ankle joints (Chang et al. 2017). Also, in severe knee osteoarthritis, not only the kinematics of the knee during gait are affected, but also that of the hip and ankle joints (Astephen et al. 2008a). In our data, PWHA showed similar joint damage at the knee and ankle joints (Table 2). However, the extent of joint movement during gait was lower at the knee level than ankle (6.3° vs. 11.6°, respectively), in proportion to the normal ROM reported during gait at slow speed (1.0 m/s) (Cruz-Montecinos et al. 2020c; Winiarski et al. 2019).

Limitations

This study has some limitations. First, joint kinetics were not assessed. Therefore, it is unclear if and how the mechanical demands were affected by the acute knee joint constraint and if these were different between the CG with the acute constraint and people with chronic knee constraint. Second, it was not possible to recruit people with chronic constraint in only the knee joint. Therefore, it is not possible to attribute the altered neuromuscular control in people with chronic knee constraint exclusively to musculoskeletal changes in the knee joint. Third, the time of artificial knee constraint exposure used in our study may not have been sufficient

for adaptations in the central nervous system. Future studies are needed to reveal if more prolonged exposure to an artificial joint constraint changes neuromuscular control of gait. Finally, because we only included men in our study, extrapolation to women should be done with caution. Because the arthropathy predominantly affects males, effects of a chronic knee constraint in women cannot be investigated in people with haemophilia.

CONCLUSIONS

We conclude that in the short-term a knee constraint does not affect the modular organization of gait, but in the long-term a knee constraint results in modular reorganization. These results indicate that merging of muscle synergies may also occur when changes in the mechanics of the musculoskeletal system is the primary cause of the motor impairment. The present results offer a new perspective on the adaptations of the central nervous system to joint restriction. These new insights may contribute to improving those medical and physical rehabilitation treatments that aim to regain the impaired walking, especially in those diseases where the motor impairment is accompanied with chronic joint constraints.

APPENDIX

The motor modules and motor primitives of the control group (with and without artificial constraint) based on the tVAF > 90 % criterion is shown in Figs. A1 and A2. For those participants with three and four synergies, a similar structure of motor modules was observed. The results of the merged synergies analysis are shown in Table A1. Only in the chronic knee joint constraint, a reorganization of synergy 1 (acceptance) and synergy 2 (propulsion) were observed. The motor modules of synergy 1 appeared to be composed of the motor modules of synergy 1 and synergy 2, also the motor modules of synergy 2 appeared to be composed of the motor modules of synergy 2 and synergy 1.

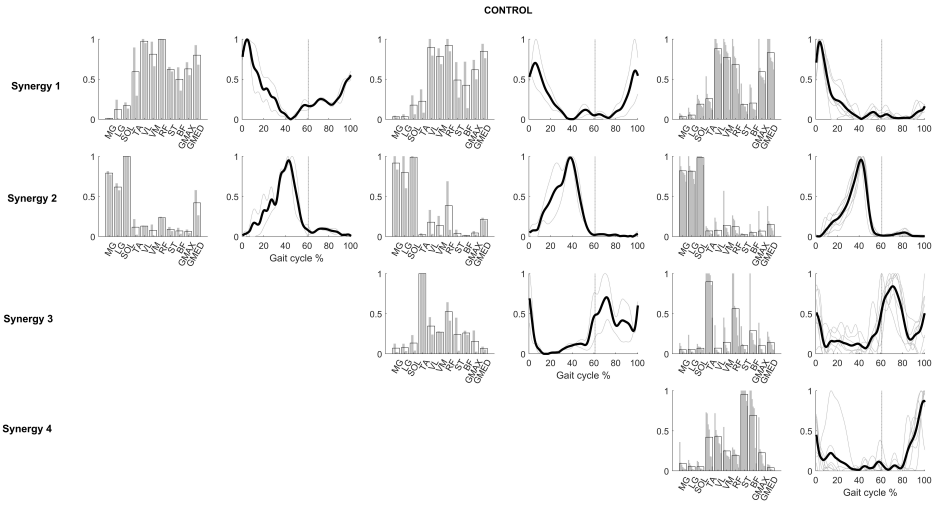


Figure A1. The average and individual data for motor modules and motor primitives of control group based on the tVAF >90% criterion. The motor primitives are plotted as a function of normalized step time (0, heel strike). The vertical dotted lines indicate the end of the stance phase. a.u, Arbitrary units; BF, biceps femoris; GMAX, gluteus maximus; GMED, gluteus medius; LG, lateral gastrocnemius; MG, medial gastrocnemius; RF, rectus femoris; SOL, soleus; ST, semitendinosus; TA, tibialis anterior; tVAF, total variance accounted for; VM, vastus medialis; VL, vastus lateralis.

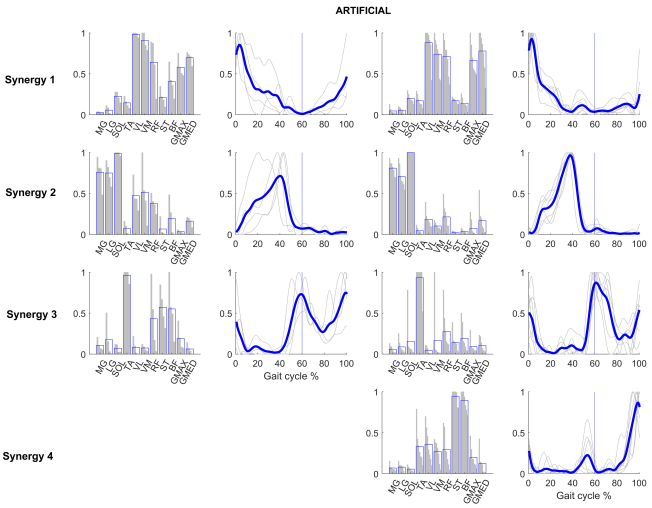


Figure A2. The average and individual data for motor modules and motor primitives of artificial constraint based on the tVAF > 90% criterion. The motor primitives are plotted as a function of normalized step time (0, heel strike). The vertical dotted lines indicate the end of the stance phase. a.u, Arbitrary units; BF, biceps femoris; GMAX, gluteus maximus; GMED, gluteus medius; LG, lateral gastrocnemius; MG, medial gastrocnemius; RF, rectus femoris; SOL, soleus; ST, semitendinosus; TA, tibialis anterior; tVAF, total variance accounted for; VM, vastus medialis; VL, vastus lateralis.

Table A1. All results of merged synergies analysis

	M1+M2			M1+M3			M1+M4			M2+M3			M2+M4			M3+M4		
	CR	SIM	%	CR	SIM	%	CR	SIM	%	CR	SIM	%	CR	SIM	%	CR	SIM	%
Chronic (n=8)																		
M1	**0.46**	**0.91**	**63***	0.06	0.78	0	0.23	0.82	13	0.50	0.70	13	0.47	0.80	25	0.13	0.67	0
M2	**0.45**	**0.90**	**50***	0.09	0.67	0	0.27	0.69	13	0.39	0.78	25	**0.45**	**0.85**	**38**	0.13	0.61	0
M3	0.19	0.57	0	0.09	0.89	13	0.17	0.58	13	0.14	0.89	25	0.16	0.61	0	0.12	0.89	0
M4	0.25	0.71	0	0.35	0.74	13	0.22	0.78	13	0.29	0.67	0	0.18	0.78	25	0.30	0.83	0
Artificial (n=11)																		
M1	0.03	0.96	0	0.10	0.96	0	0.04	0.96	9	0.18	0.55	0	0.08	0.70	0	0.18	0.70	0
M2	0.01	0.97	0	0.11	0.39	0	0.16	0.42	0	0.09	0.94	0	0.11	0.96	0	0.06	0.40	0
M3	0.19	0.48	0	0.07	0.83	0	0.09	0.54	0	0.14	0.88	0	0.16	0.58	0	0.11	0.84	0
M4	0.11	0.55	9	0.22	0.67	0	0.08	0.87	0	0.11	0.63	0	0.07	0.87	0	0.19	0.89	18

To assess whether one synergy may be reconstructed from a pair of merged synergies of the control group, two criteria were adopted:*1*) coefficient of the reconstruction (CR) > 0.3 and *2*) similarity (SIM) > 0.8. The merged results with two criteria met are printed in bold. If two criteria were met, the frequency of appearance between groups were tested (chronic vs. artificial). *Statistical significance ($P < 0.05$). M, motor module.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

SUMMARY OF FINDINGS

The overall aim of my thesis was to investigate the neural control of gait in PWHA. The hypothesis of my thesis was that neural control is affected in PWHA and that these changes are associated with the severity of joint damage and chronicity of the joint constraint. **In Chapters 2 and 4**, through the assessments of muscle activation patterns of single muscles, coordination between antagonistic muscle pairs, and muscle synergy analysis, we confirmed the hypothesis that the neural control of gait is affected in PWHA. In **chapters 3 and 5**, we confirm that the altered neural control of gait in PWHA is associated with the severity of joint damage and chronicity of joint constraint.

In Chapter 2, based on muscle synergy analysis, we showed that the neural control of gait in PWHA with varying levels of restriction in joint motion differs from healthy controls. Specifically, we found higher co-activation between knee flexors/extensors and between knee extensors/ankle plantar flexors. In this chapter, the WALK-DMC index, which is based on the total EMG variance explained by one synergy, was described for the first time as an alternative metric to assess the neural control of gait in PWHA. This population showed a reduced complexity of neural control compared to the healthy group. Using the Walk-DMC index, we showed also that the neural control of gait is affected more in PWHA with multi-joint damage (knee and ankle) than single-joint damage (only ankle).

In Chapter 3, based on the calculation of the Walk-DMC index from 11 leg muscles in PWHA with varying levels of restriction in joint motion, we showed that the Walk-DMC index in PWHA was associated with the degree of joint damage, knee flexion contracture, and pain. PWHA with altered neural control of gait (i.e., Walk-DMC below 80 points) have experienced arthropathy for a longer time and have more pain. We also found that the assessment of the Walk-DMC is dependent on the number of muscles included. The Walk-DMC based on the EMG signal of five to eight muscles was different than that based on the EMG of 11 muscles. We concluded that the Walk-DMC index may be used as an additional assessment tool to monitor the arthropathy progression, and the assessment of the Walk-DMC index is sensitive to the number of muscles included for EMG measurements.

In **Chapter 4**, we recruited PWHA with a mild restriction in joint motion (i.e., range of motion of the knee >60° and of the ankle >20°). Waveforms of EMG and sagittal plane joint angles were compared with healthy controls. In addition, the timing of EMG onset-offset and coordination between antagonistic muscle pairs during gait were compared between PWHA and healthy controls. The main results were that in PWHA the biarticular plantar flexors were excited earlier and the hip extensors and knee flexors/extensors were turned off later during the stance phase compared to that of controls. In addition, the sagittal plane range of motion in hip, knee and ankle joints was lower in PWHA. We concluded that the neural control of individual leg muscles, coordination between antagonistic muscle pairs around the knee and ankle, and sagittal kinematic of the hip, knee, and ankle during gait in PWHA differs substantially from control subjects.

In **Chapter 5**, we recruited healthy controls and PWHA with a severe chronic knee joint constraint (range of motion of the knee < 20°). To mimic the knee constraint

of PWHA, healthy controls walked with a brace to constrain knee joint movement to 20°. Using a muscle synergy analysis, we showed that short-term exposure to knee constraint in controls does not affect the modular organization of gait. However, long-term exposure to a knee constraint in PWHA resulted in a merging of acceptance and propulsion synergies. We concluded that the changes in neural control in PWHA can be explained by the chronicity of the knee joint constraint and not by the knee joint constraint itself.

CLINICAL AND PHYSIOLOGICAL CHARACTERISTICS OF ALTERED NEURAL CONTROL OF GAIT IN PWHA

Haemophilic arthropathy may result in several changes in the musculoskeletal system that can result in motor impairment. Most of the studies have assessed neuromuscular control in PWHA during static conditions (Göhler et al. 2014; Kurz et al. 2011, 2012). However, little was known about neural control of gait in PWHA. **In Chapters 2 and 5**, using the muscle synergy analysis, we found that PWHA have less complex neuromuscular control (i.e., higher VAF1 and lower Walk-DMC index) and altered modular organization for the acceptance and propulsion synergies. In a secondary analysis in **Chapter 2**, I applied a cluster analysis to identify sub-groups of PWHA at different levels of joint damage in the knee and ankle assessed by HJHS score. The results indicate that on average PWHA with multi-joint impairment have a lower value (average of 80 points) on the Walk-DMC index than PWHA with single-joint impairment. In **Chapter 3,** we observed an altered Walk-DMC index (i.e., < 80 points) in 13 out of 22 PWHA. PWHA with altered neural control of gait showed more years with arthropathy, more pain, more knee flexion contracture, and more joint impairment.

Considering the multiple impairments of the musculoskeletal system in PWHA, it is not possible to attribute the altered neural control to a single clinical factor. Thus, several factors may contribute to the changes in the neural control of gait. For instance, it is well documented that a) pain, b) severity of joint damage, and c) aging alters neural control.

Regarding pain, several studies have reported that experimental-induced pain reduces the motor drive to painful muscles and its synergists during single-joint isometric tasks (Astephen et al. 2008b; Hodges 2011; Tucker and Hodges 2010). However, during gait, specific simulated pain in the leg or back muscles did not affect the organization of modular control (Van Den Hoorn et al. 2015). The differences between the isometric and dynamic tasks have been attributed to the higher adaptability of gait, which involves multiple degrees of freedom, and thus various possibilities for adaptation in muscle coordination (Van Den Hoorn et al. 2015). Therefore, acute pain alone may not be sufficient to modify modular control during gait. In contrast to acute pain, joint damage in combination with chronic pain (people with knee osteoarthritis) was reported to result in a reorganization of modular control during gait (Kubota et al. 2021). This reorganization was characterized by merged reception and propulsion synergies. The same authors proposed that the merging of these two synergies can be used as a biomarker of joint damage. Interestingly, we observed the same reorganization in the modular control in PWHA with a chronic knee constraint **(Chapter 5)**. Hence, the modular reorganization of gait may be associated with the severity of joint damage.

Like PWHA, aging is accompanied by changes in neural (e.g., altered proprioception and decreased postural control) and mechanical factors (e.g., loss of knee extension and decreased muscle strength) (Aoyagi and Shephard 1992; Kubota et al. 2021;

Michalska et al. 2021; Woollacott et al. 1986). Changes in both factors may affect the neural control of gait and increase the risk for the development of knee osteoarthritis (Campbell and McGonagle 2021; Hodges et al. 2016; Kubota et al. 2021). However, unlike osteoarthritis, haemophilic arthropathy can occur in young adults, even children. Therefore, other factors such as the repetitive intraarticular bleeding, the time of exposure to joint damage and joint constraints (see below) should be also considered as factors that may change the neural control of gait in PWHA **(Chapters 3 and 5).**

In Chapter 4, we showed that the timing of activation of the individual leg muscles is altered in PWHA. Specifically, we investigate the activation patterns of muscles around the hip, knee, and ankle joints. PWHA showed longer activity of hip-knee flexors and extensors and earlier activity of biarticular ankle plantar flexors than healthy controls. Similar patterns have been reported in people with knee osteoarthritis (Astephen et al. 2008b; Mills et al. 2013a). The longer activation of hip and knee extensors during the stance phase may be a neural strategy to decrease pain sensation and increase joint stability during the stance phase (Mills et al. 2013a; Rutherford et al. 2012). However, increased co-contraction may lead to increased joint loading (Hodges et al. 2016; Shelburne et al. 2006) and increased metabolic cost of gait (Griffin et al. 2003; Rutherford et al. 2012). A longer duration of co-contraction around the knee correlates positively with annual percent loss of medial tibial cartilage volume (Hodges et al. 2016). In PWHA, joint damage has been attributed to repetitive intraarticular bleeding and chronic synovitis. However, the potential contribution of altered neural control in the progression of joint deterioration in PWHA has not been studied yet. Future studies are needed to address this hypothesis.

In chapter 4, we also compared the sagittal plane joint angles of the hip, knee, and ankle joints between PWHA and healthy controls. Despite that PWHA had a passive ROM > 60°, which corresponds to the required ROM during gait, a reduced knee ROM was observed compared to healthy controls. The reduced knee ROM may be explained by increased co-contraction. The increased co-contraction between knee extensors and plantar flexors during the stance observed in PWHA may have reduced the peak knee flexion angle during the swing phase by reducing the angular velocity of the shank during toe-off (Goldberg et al. 2003; Piazza and Delp 1996). The increased co-contraction by PWHA may be considered as a strategy to increase stability and reduce the pain, at the expense of constraint the range of motion.

NEURAL CONTROL OF GAIT AND LONG-TERM EXPOSURE TO JOINT CONSTRAINT

Several neurological and musculoskeletal diseases affect joint ROM. Joint constraints in the lower limb interfere with many essential activities of daily living, such as walking, stair climbing, or standing up, affecting people's ability to function independently and their quality of life. The restriction of joint movement during gait is well documented for PWHA. However, little was known about how joint constraints affect the neural control of gait in PWHA.

In Chapter 5, we reported that modular reorganization in PWHA is the result of a long-term adaptation to a knee joint constraint, because such reorganization was not found for acutely restricting knee ROM. These results indicated that the modular reorganization of gait may also occur when the musculoskeletal system is the primary cause of motor impairment. In **Chapter 3**, we reported that PWHA with altered Walk-DMC index had more years with arthropathy than PWHA with normal Walk-DMC. These results agree with studies in rats, where one month of joint

constraint results not only in musculoskeletal changes but also in changes in the CNS (i.e., spasticity, hyperexcitability of the spinal network, and changes in the organization of somatosensory cortex) (Canu et al. 2022; Delcour et al. 2018b). The results presented in **Chapters 3 and 5** indicate that chronic exposure to a joint constraint alters neural control of gait in PWHA. Thus, exposure duration to joint constraint should be considered a factor that may affect the neural control of gait in PWHA. This is relevant since when these changes in the nervous system appear, it may be more challenging to recover neural control of gait.

CLINICAL IMPLICATIONS OF NEURAL CONTROL ASSESSMENT IN PWHA

Haemophilic arthropathy has been classified as a musculoskeletal disease. However, based on the result of my thesis, haemophilic arthropathy should be considered a disease that may affect the neuro-musculoskeletal system. Identifying those PWHA with altered neural control of gait may help design a more personalized medicine and rehabilitation. For example, when clinicians plan a total knee replacement in PWHA with chronic knee constraint, long exposure to joint restriction can make it difficult to regain a normal range of motion due to impaired gait control (**Chapters 3 and 5**). Therefore, recovery of passive knee ROM after total knee replacement in PWHA should be combined with gait re-education to encourage active use of ROM, especially in those PWHA with knee flexion contracture (Atilla et al. 2012; Kubeš et al. 2018; Oyarzun et al. 2020).

As mentioned before, long-term exposure to joint constraints may result in persistent changes in the CNS. Therefore, clinicians should consider that delaying the total replacement surgeries may expose PWHA to changes in the neural control of gait, affecting the results of surgery and rehabilitation.

Passive ROM exercises are usually prescribed to restore the ROM in PWHA. However, the passive exercises may not be sufficient to restore ROM during gait due to the changes in neural control of gait (modular reorganization). Consequently, restoring the ROM during gait should also involve therapeutic exercises (i.e., neuromuscular exercises and biofeedback) to reduce co-contraction and transfer the improvements to daily life activities.

Regarding the clinical implications of assessing the neural control of gait, the Walk-DMC index can be used as an additional assessment tool to monitor disease progression in PWHA and the effect of orthopedic surgeries. An example of the latter is the following case report, in which the Walk-DMC index was assessed at 1 m/s of walking velocity before and after two years of total knee replacement (Figure 4). The case consisted of a follow-up of a patient with severe haemophilia (age 28 years, body mass index 20.8 kg/m^2), severe pain (VAS 8), knee flexion contracture of 30° (assessed passively), and a history of knee arthropathy of 12 years. The patient received physiotherapy (strength exercises, proprioception exercise and muscle stretching) six months before and one year after the surgery. After two years, we observed that despite that the patient had no pain (VAS 0) and knee flexion contracture was significantly reduced (5°), neural control was not returned to a normal level (i.e., Walk-DMC <80) (Figure 4).

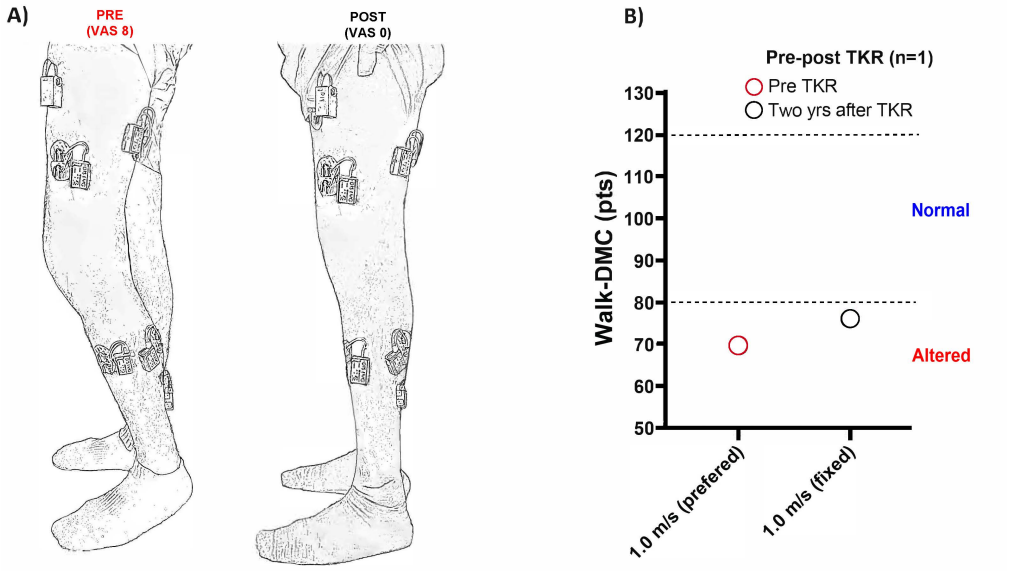


Figure 4. Picture of the bipedal posture before and after two years of total knee replacement (TKR) in one adult with severe haemophilia A (total HJHS of 42 points, limb HJHS 25 points). Visual analogue scale (VAS). **B.** Changes in the Walk-DMC at fixed velocity (1 m/s). Two years after total knee replacement, the Walk-DMC was still lower than 80 points, indicating that the neural control of gait was not fully recovered after two years of surgery (data not published).

The improvement of clinical outcomes (e.g., increase ROM and decrease knee flexion contracture) after total knee replacement in PWHA is well reported (Fenelon et al. 2022). However, it is unknown how neural control is affected by total knee replacement. Studies on people with osteoarthritis have reported that muscle activity patterns differ from controls even five years after total knee and ankle replacement (Hatfield et al. 2021; De la Fuente et al. 2018b). One possible explanation for these findings is that chronic exposure to joint damage may involve CNS reorganization, implying that recovery of neural control of gait is not necessarily automatic after the total joint replacement (di Laura Frattura et al. 2019).

Detecting if the altered neuromuscular control is normalized or not may also provide targets for conservative interventions (e.g., proprioception exercises, balance training, joint mobility and strength exercises) before and after surgery (Lobet et al. 2008), especially in those PWHA with long term-exposure to a joint constraint.

The altered neural control of gait is usually reported in neurological diseases such as stroke and cerebral palsy (Bekius et al. 2020; Van Criekinge et al. 2020). Neurological diseases may also result in changes of the musculoskeletal system, including pain and joint contractures (Sackley et al. 2008). However, recently it has been reported that simulating cerebral palsy contractures in healthy people does not affect the modular organization (Spomer et al. 2022). The latter suggests that altered synergies are not primarily a reflection of joint constraints in cerebral palsy but likely capture the underlying altered organization of the CNS (Spomer et al. 2022). These findings agree with the results of **Chapter 5**, where the artificial knee joint constraint did not mimic the altered neural control of chronic joint constraint in PWHA. Thus, haemophilic arthropathy should be considered a disease that affects not only the musculoskeletal (i.e., joint restrictions, muscle atrophy) but also the neural systems (i.e., modular reorganization).

METHODOLOGICAL CHALLENGES OF NEURAL CONTROL ASSESSMENT

In my thesis, two different EMG-based approaches were used to study the neural control of gait: the activation patterns of single muscles, and muscle synergies. Both types of analysis can be used in a complementary way. The analysis of single muscles may help to understand how the CNS changes the timing and amplitude of the activation pattern of a single muscle in response to changes in mechanical load

(e.g., walking speed, inclination level). At the same time, the muscle synergy analysis may serve to understand how the CNS controls the timing and amplitude of a group of muscles. The interpretations of both approaches are also different. Changes in amplitude and temporal pattern of a single muscle indicate a change in the load of the muscle (Nichols 2018). In contrast, the changes in a modular organization may imply an altered supraspinal input and spinal circuit, resulting in changes in the complexity of neuromuscular control (Tresch and Jarc 2009).

One marked challenge of neural control assessment is the interpretation by clinicians. In comparison to a comprehensive synergy analysis (i.e., number of synergies, the variance accounted for, motor modules, and motor primitives), the Walk-DMC index has been proposed as a summary metric of neural control of gait that may be interpreted rapidly by clinicians and researchers. For its correct application, clinicians and researchers must consider that the Walk-DMC index is sensitive to the number of muscles, number of gait cycles and walking velocity (Bekius et al. 2020; Oliveira et al. 2014; Shuman et al. 2018; Steele et al. 2013). Thus, a standardized protocol is needed to monitor the changes in neural control (see below).

In **Chapter 3,** our results indicated that the EMG of a minimum of eight leg muscles needs to be included in the assessment of the Walk-DMC. If less than eight muscles are included, the complexity of neural control will be underestimated. In PWHA, the WALK-DMC we observed that are less affected by the number of muscles due to the higher co-contraction among leg muscles (Figure 5). However, increasing the number of muscles in healthy controls decreases the tVAF1, augmenting the differences with PWHA (Figure 5).

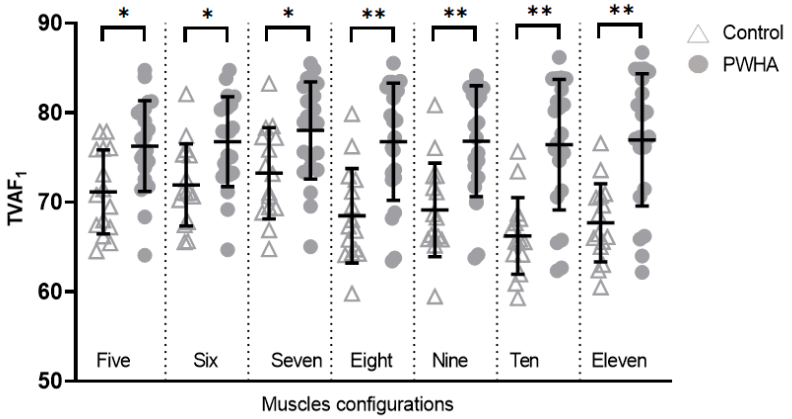


Figure 5. TVAF1 between the control healthy group (n=15) and PWHA (n=22) using different muscle configurations (five to eleven leg muscles). For the representation of each configuration, see Table 1 in Chapter 3. The data are presented as mean and 95% confidence interval. *p<0.05. **p < 0.005. (Data not published).

The number of gait cycles may also affect the calculation of tVAF1 (Shuman et al. 2016). Considering the variability of muscle activation patterns, especially in clinical populations, twenty gait cycles have been suggested to be enough to have a representative variability of muscle activation patterns (Shuman et al. 2016). Walking velocity should also be considered to calculate the Walk-DMC index. Increasing walking velocity may decrease the co-contraction, and hence decreased the tVAF1 affecting the Walk-DMC index. In cerebral palsy, Walk-DMC has been significantly associated with changes in walking speed, where a change of 10% in walking velocity corresponds to a preoperative difference of 16 pts in the Walk-DMC (Shuman et al. 2018). In all my experiments, the control group's velocity was constrained to the comfortable velocity of the PWHA (1 m/s).

In summary, the assessment of neural control of gait in clinical scenarios should take the following points into account. First, the walkway where the EMG data is collected must have enough length to collect at least 20 cycles continuously. Second, the Walk-DMC index may be a good alternative to assess the neural control of gait due that clinicians are not familiar with muscle synergy analysis. The EMG activity of minimally eight muscles in the leg should be measured. Third, for clinical purposes, to follow patients at multiple time points, the velocity should be kept constant at each measurement, and matched with the target clinical group.

LIMITATIONS

The results presented in each of the chapters of my thesis are not without limitations. The first limitation is the absence of information about joint kinetics. Therefore, it is unclear if the mechanical loads were different in PWHA and how the kinetics were related to the changes in neural control of gait. Future studies should include the assessment of joint moments. Secondly, the muscular and non-muscular (i.e., tendon, nerve and fascia) mechanical properties of joints were not assessed. Repetitive intermuscular and intraarticular bleeding may increase the mechanical interaction between muscles. Enhanced mechanical interaction between synergistic muscles has been shown to alter intermuscular coordination (Bernabei et al. 2017). Unfortunately, little is known about the changes in muscular and non-muscular mechanical properties in PWHA (Cruz-Montecinos et al. 2015, 2019b)**.** We know so far that ankle joint damage is associated with a reduction in Achilles tendon stiffness in PWHA (Cruz-Montecinos et al. 2019b). However, the results were not compared with a healthy control group. Future studies are needed to understand the interaction between the muscular and non-muscular mechanical properties and changes in the neural control of gait in PWHA.

The altered proprioception (i. e., angle-reproduction) may also result from joint damage in PWHA (Hilberg et al. 2001), which may alter the peripheral feedback to the spinal cord and the brain, affecting the spinal circuit, and resulting in changes in the duration and magnitude of muscle activity (Frigon et al. 2022). We did not assess the proprioception. Future studies are needed to understand the association between proprioception and neuromuscular control of gait in PWHA.

Furthermore, we did not assess psychological factors, such as kinesiophobia and catastrophism (Ucero-Lozano et al. 2021). These variables may affect neural control and should be addressed in future studies (Kantak et al. 2022; De Oliveira Silva et al. 2020). Finally, the intraday reproducibility of EMG, muscle synergy analysis, and Walk-DMC index is not available for PWHA but has been shown to have a good reproducibility in other clinical populations (e.g., cerebral palsy) (Shuman et al. 2016). Researchers and clinicians who aim to study the effects of therapeutic interventions (physiotherapy or orthopaedic surgery) on the neural control of gait in PWHA should consider generating studies that address the reproducibility of single-leg EMG analysis, muscle synergy analysis, and Walk-DMC index.

Finally, the generalizability of our findings should be considered with caution. Although the sample size of the studies was justified based on power analyses, the PWHA sample in each chapter was small (Appendix table 1). Furthermore, access to medical treatments and physical therapy may vary between countries, affecting the disease progression. Future multicentric studies are needed to compare the neural control of gait in PWHA between countries.

FUTURES DIRECTIONS AND CLOSING REMARKS.

Before starting my PhD project, very little was known about the changes in neuromuscular control in PWHA. Previous studies reported that PWHA have reduced muscle force, altered proprioception (Hilberg et al. 2001), and impaired balance control (Cruz-Montecinos et al. 2017, 2020b; Deschamps et al. 2018; Gallach et al. 2008). Regarding neural control, the focus has been primarily on neural control of leg muscles during standing, not gait (Kurz et al. 2011; Seuser et al. 2018). Furthermore, several papers have reported that PWHA show altered joint kinematics and kinetics during gait (Eerdekens et al. 2020; Lobet et al. 2010, 2013; Putz et al. 2020; Stephensen et al. 2012). In addition, one study conducted by Lobet et al. 2013 found that the metabolic cost is increased in PWHA, and the metabolic cost was highly correlated to a loss in ROM of ankle, knee and hip joints. A possible explanation for the high energy demand observed in PWHA is the increased co-contraction during walking to maintain balance (Lobet et al. 2013). However, the hypothesis that PWHA moves with greater co-contraction than healthy subjects had not been tested prior to my PhD project.

How to improve the neural control of gait in PWHA?

Recovering to normal neural control of gait is challenging for PWHA and various other musculoskeletal and neurological pathologies involving restrictions in joint ROM. Typically the therapeutic approaches in PWHA include joint mobility exercises, muscle strengthening exercises, and balance exercises (Strike et al. 2016). However, these approaches may not be enough to guarantee the restoration of neural control of gait. Since neural control of gait is affected in PWHA, the CNS may need extra information about controlling the muscles. A potential approach

that may help PWHA to understand how they control their muscles is biofeedback. Effects of biofeedback have not been tested in PWHA, but in several other patient groups (e.g., incomplete spinal cord injuries, stroke and cerebral palsy)(Booth et al. 2019; Petrofsky 2001).

In patients with incomplete spinal cord injuries, feedback of EMG activity from gluteus medius muscle during walking for two months was shown to reduce the pelvic tilt during gait (Petrofsky 2001). Another study on stroke patients reported that 12 sessions of treadmill walking with feedback of EMG activity of the tibialis anterior and gastrocnemius lateralis muscles of the affected side increased gait speed, ankle power generation at push-off, and reduced the knee extensor moment (Aiello et al. 2005). In cerebral palsy, different modalities of feedback (step length, knee extension, and ankle power) have been shown to result in subtle changes in muscle synergies (Booth et al. 2019). The latter suggests that long-term feedback may be needed to modify the organization of the CNS.

Another potential technique that may help normalize the neural control of gait is functional electrical stimulation (FES)(Ambrosini et al. 2012, 2020). FES provides afferent inputs to the CNS for re-learning the proper motor coordination by stimulating legs muscles according to a physiological stimulation strategy derived from EMG activation patterns from a healthy matching group (Ambrosini et al. 2012, 2020). The combination of treadmill training and FES of ankle muscles has improved walking speed and energy efficiency post-stroke (Awad et al. 2014; Reisman et al. 2013). Although biofeedback and FES appear to have great potential for PWHA, future studies are needed to confirm the effects of long-term training in randomized controlled clinical trials.

Finally, I would like to remark that the neural control of gait should be addressed not only as a goal to improve gait economy or improve post-surgery clinical outcomes. It may also be addressed as a potential determinant of intraarticular load. It is clear that intraarticular bleeding is the principal factor in arthropathy's physiopathology (van Vulpen et al. 2018). However, once the joint damage is established, altered neural control may also contribute to the arthropathy progression. Future studies are needed to understand the role of altered neural control in advancing joint deterioration in PWHA.

CONCLUSIONS

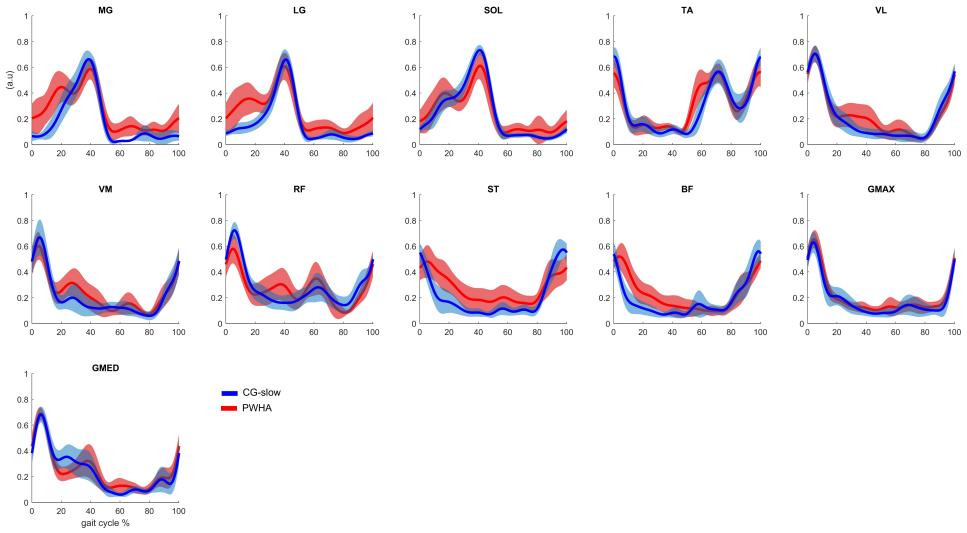
My thesis contributes to understanding the consequences of haemophilic arthropathy for the neuromechanics of gait. The findings of my thesis indicate that neural control of gait is affected in PWHA, and the changes in the neural control of gait are associated with joint damage, pain and chronicity of the joint constraint. From a scientific perspective, the changes in the neural control of gait in PWHA implicate altered activity patterns of single leg muscles and a modular reorganization of gait. From a clinical perspective, my thesis gives a new perspective on how to monitor disease progression in PWHA using the Walk-DMC index—providing new perspectives to improve the therapeutic interventions that aim to recover gait in PWHA.

Appendix

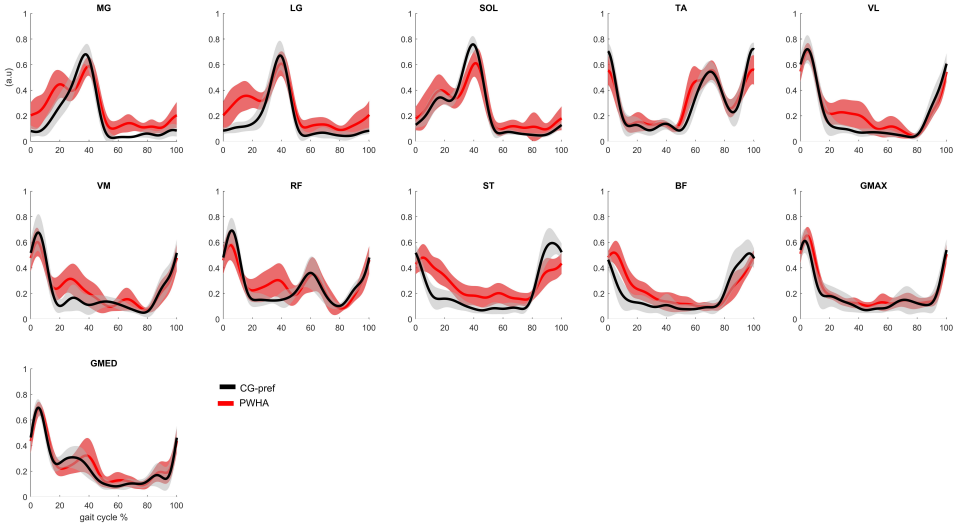
Table 1. Participants in each chapter.

	PWHA					CONTROL			
PA	Chapt. 2 (n=13)	Chapt. 3 (n=22)	Chapt. 4 (n=14)	Chapt. 5 (n=8)	PA	Chapt.2 (n=13)	Chapt. 3 (n=15)	Chapt. 4 (n=13)	Chapt. 5 (n=11)
1	✓	✓	✓		1	✓	✓	✓	
2	✓	✓	✓		2	✓	✓	✓	✓
3	✓	✓	✓		3	✓	✓	✓	✓
4	✓	✓	✓		4	✓	✓	✓	✓
5	✓	✓	✓		5	✓	✓	✓	
6	✓	✓	✓		6	✓	✓	✓	✓
7	✓	✓	✓		7	✓	✓	✓	✓
8	✓	✓	✓		8	✓	✓	✓	
9	✓	✓	✓		9	✓	✓	✓	✓
10	✓	✓	✓		10	✓	✓	✓	✓
11	✓	✓	✓		11	✓	✓	✓	✓
12	✓	✓	✓		12	✓	✓	✓	✓
13		✓	✓		13	✓	✓	✓	
14		✓	✓		14		✓		✓
15	✓	✓		✓	15		✓		✓
16		✓		✓					
17		✓		✓					
18		✓		✓					
19		✓		✓					
20		✓		✓					
21		✓		✓					
22		✓		✓					

The symbol ✓ indicates involvement in the study. Participants (PA). Chapter (Chapt). People with haemophilic arthropathy (PWHA).



Appendix figure 1. Surface electromyography of leg muscles of chapter 4. Data are plotted as a function of normalized step time (0, heel strike) and expressed as mean with 95% confidence interval. Control group preferred velocity (CG-slow, blue). People with haemophilic arthropathy (PWHA, red).



Appendix figure 2. Surface electromyography of leg muscles of chapter 4. Data are plotted as a function of normalized step time (0, heel strike) and expressed as mean with 95% confidence interval. Control group preferred velocity (CG-pref, black). People with haemophilic arthropathy (PWHA, red).

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SUMMARY

Haemophilia is a bleeding disorder caused by a deficiency of coagulation factors VIII or factor IX. People with severe haemophilia may have spontaneous bleeding events or bleeding in response to minor trauma; most of the events occur in the joints and muscles. In people with haemophilia, the most frequent clinical manifestation is haemophilic arthropathy, which results from repetitive intraarticular bleeding and inflamed synovial membrane. Haemophilic arthropathy often results in chronic pain and joint impairment, affecting motor function and quality of life. Previous research has focused predominantly on the musculoskeletal aspects of haemophilia, with a few studies addressing the potential effects of arthropathy on the central nervous system. **In chapter 1**, I provide a general perspective about the impact of haemophilia on the musculoskeletal system, the current gaps in knowledge in the neural control of gait in haemophilic arthropathy, and how to assess the neural control of gait. The overall aim of my thesis was to investigate the neural control of gait in PWHA. The specific aims were: i) to assess changes in EMG activity patterns of single muscles, ii) to assess changes in modular organization of gait, iii) to investigate the association between clinical measures of musculoskeletal function and measures of modular organization and iv) to elucidate the mechanisms driving the changes in neural control. I hypothesized that neural control of gait is affected in PWHA, and the changes in neural control of gait are associated with the degree of joint damage and chronicity of the joint constraint. Gait was selected because the knees and ankles are the most affected joints in PWHA. The core of my thesis is investigating neural control by studying EMG activity patterns of single muscles and/or muscle synergies, as well as their interaction with joint kinematics and clinical outcomes. For this purpose, EMG

activity of muscles crossing hip, knee and ankle as well as the lower limb kinematic, and clinical measures (joint health, pain, walking velocity) were assessed. **In chapter 2,** based on muscle synergy analysis we investigate the changes in the complexity of neuromuscular control during gait in PWHA with varying levels of arthropathy severity (mild-severe joint restrictions) compared to a healthy control group. This chapter described for the first time the effect of haemophilic arthropathy on neuromuscular control of gait. **In Chapter 2**, we showed that the neural control of gait in PWHA with varying levels of arthropathy severity differs from healthy controls. Specifically, in terms of higher co-activation between knee flexors/extensors and between knee extensors/plantar flexors. In this chapter, the WALK-DMC index is described for the first time as an alternative metric to assess the neural control of gait in PWHA. PWHA showed a reduced complexity of neural control compared to the healthy group, independent of the walking velocity of the control group (slow and preferred). Using the Walk-DMC index, we also showed that the neural control of gait is affected more in PWHA with multi-joint damage than single-joint damage. **In Chapter 3** we investigate the association between clinical outcomes (joint health, pain and the degree of knee flexion contracture) and Walk-DMC in PWHA with varying levels of arthropathy severity (mild-severe joint restrictions). In addition, the clinical outcomes of PWHA with normal and altered neural control of gait were compared. Based on the calculation of the Walk-DMC index from 11 leg muscles, we identified those PWHA with altered neural control of gait (i.e., Walk-DMC below 80 points). We showed that the Walk-DMC index in PWHA was associated with the degree of joint damage, knee flexion contracture, and pain. PWHA with altered neural control of gait also have experienced arthropathy for a longer time, more pain, and more impaired joints. We also found that the Walk-DMC assessed based on the EMG signal from five to eight muscles was different than that based on the EMG of 11 muscles. We

conclude that the Walk-DMC index may be used as an additional assessment tool to monitor the arthropathy progression, and the assessment of the Walk-DMC index is sensitive to the number of muscles used for EMG measurements. **In Chapter 4**, we investigate the changes in the neural control of individual muscles and joint kinematics in PWHA with mild joint restriction compared with healthy controls using statistical parametric mapping on the time series. In addition, the timing of EMG onset-offset and coordination between antagonistic muscle pairs during gait were compared between PWHA and healthy controls. The main results were that PWH excited earlier on the biarticular plantar flexors, and the hip extensors and knee flexors/extensors showed later offset during the stance phase compared to controls. In addition, the sagittal range of motion in hip, knee and ankle joints was lower in PWHA. In this chapter, we conclude that the neural control of individual leg muscles (amplitude and temporal on-off), coordination between antagonistic muscle pairs around the knee and ankle, and sagittal kinematic of the hip, knee, and ankle during gait in PWHA differs substantially from control subjects. **In Chapter 5,** we assessed the effects of short-term (minutes) and long-term exposure (years) to severe knee joint constraints on modular organization of gait. In this chapter, we investigate how the CNS responds to a knee joint constraint in healthy controls (short-term) and has adapted in PWHA with long-term exposure to a joint constraint. In this chapter we recruited healthy controls and PWHA with a severe chronic knee joint constraint (range of motion of the knee < 20 °). The healthy subjects also walked with a constraint limiting knee joint movement to 20°. Using a muscle synergy analysis, we showed that short-term exposure to knee constraint in controls does not affect the modular organization of gait. However, long-term exposure to a knee constraint in PWHA resulted in a merging of acceptance and propulsion synergies. Overall, these results indicate that the short-term knee constraint does not affect the modular organization of gait, but the long-

term knee constraint results in modular reorganization. In this chapter, we concluded that the changes in neural control in PWHA can be explained by the chronicity of the knee joint constraint and not by the knee joint constraint itself. **In chapter 6,** we discussed the results of chapters focused on clinical and physiological perspectives of the altered neural control of gait in PWHA. In addition, we discuss how the evaluation of neural control through the Walk-DMC index can be an alternative to monitoring the motor impairment in PWHA. Based on our results, we also discussed on how to improve the outcomes of physical therapy and surgical interventions that aimed to improve the locomotion in PWHA. In conclusion, neural control of gait is affected in PWHA, and the changes in the neural control of gait are associated with joint damage, pain and chronicity of the joint constraint. From a scientific perspective, the changes in the neural control of gait in PWHA implicate altered activity patterns of single leg muscles and a modular reorganization of gait. From a clinical perspective, my thesis gives a new perspective on how to monitor disease progression in PWHA using the Walk-DMC index—providing new perspectives to improve the therapeutic interventions that aim to recover gait in PWHA.

LIST OF PUBLICATIONS

Publications as part of this compendium thesis

Cruz-Montecinos C, Pérez-Alenda S, Cerda M, Maas H. Neuromuscular control during gait in people with haemophilic arthropathy. Haemophilia. 2019 Mar;25(2): e69-e77. *doi: 10.1111/hae.13697.*

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Cruz-Montecinos C, Pérez-Alenda S, Cerda M, Maas H. Neuromuscular control during gait in people with haemophilic arthropathy. (2019). XXVII Congress of the International Society of Biomechanics. (Oral presentation)

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Amsterdam Movement Sciences conducts scientific research to optimize physical performance in health and disease based on a fundamental understanding of human movement in order to contribute to the fulfillment of a meaningful life.